Gum Guaiac #113 10/23/73

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M O N O G R A P H

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G U M G U A I A C

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INFORMATICS INC.
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GUM GUAIAC

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GUM GUAIAC

Summary

Gum guaiac, the resin from the wood of Guajacum officinale L. or G. sanctum L., has been known to medicine for hundreds of years. In the 1700's it was considered a cure for gout, dropsies, "cutaneous foulnesses", catarrhs, ulcerations, gleet, and gonerrhea. In the early 1900's, doctor's prescribed gum guaiac for syphillis. The gum was believed to be a "sweetener and cleanser of the blood" and a diaphoretic; it was used for "cleansing the joints" and "warming and strengthing the nerves". These claims have since been successfully refuted, and gum guaiac has found extensive use as an antioxidant in foods (27).

Very little is known concerning the absorption and metabolism of gum guaiac. In 1938 Johnson et al. (27) reported that little or none of gum guaiac ingested by dogs was absorbed. In vitro results led them to conclude that the gum is partially destroyed in the colon. However, their results were quite inconsistant, partly because their quantitative test for gum guaiac had an estimated accuracy of 50%.

A diet containing 5 g of gum per 100 g of lard resulted in slightly decreased fat absorption in rats; a slight cathartic action was noted (27).

In 1970, the Joint FAO/WHO Expert Committee on Food Additives (28) stated that a specification could not be developed for gum guaiac owing to lack of information and consequently the earlier decision was confirmed.

Few short-term studies have been performed on gum guaiac. Young male rats were found to tolerate a level of 0.5% gum guaiac for 6 months; their growth rate was approximately 85% that of control rats (33). No untoward effects were discovered in the lungs, kidneys, livers, and spleens of 5 cats fed up to 1.0 g of the gum for 34-117 weeks (27). Five dogs remained healthy on a similar regimen (27).

Normal hematology, kidney function, and body weight were reported by Johnson et al. (27) in human subjects who ingested 0.05 to 0.10 g of gum guaiac for 18 to 104 weeks.

Two long-term studies were available for review. Lehman et al. (33) cited a study from Bieter (1949) in which rats were fed 0.5% gum guaiac for 2 years without pathological damage. Johnson et al. (27) fed 3 successive generations of rats 0, 0.05, 0.5 and 5.0 g gum guaiac per 100 g of lard throughout their lifetime. The histolo-

gy and reproductive parameters appeared normal for sample rats.

Lehman et al. (33) reported the oral LD_{50} of gum guaiac in mice to be greater than 2,000 mg/kg, in rats 5000 mg/kg, and 1120 mg/kg for guinea pigs. Jenner et al. (25) agreed with the 5000 mg/kg figure for rats.

GUM GUAIAC

Chemical Information

I. Nomenclature

A. Common Name

1. Gum Guaiac
2. Resin Guaiac
3. Guaiacum

B. Chemical Name

Not Applicable

C. Trade Name

None

D. Chemical Abstracts Registry Number

PM9000297

II. Empirical Formula

Not Applicable

III. Structural Formula

Gum guaiac consists of 70% alpha and beta-guaiaconic acids, about 11% guaiacic acid, related compounds and guaiaretic acid, and 15% vanillin, guaiac yellow, guaiac saponin (guaiacin).

IV. Molecular Weight

Not Applicable

V. Specifications

Food Chemicals Codex

Alcohol-insoluble residue	Not more than 15%
Melting range	Between 85° and 90°.
Limits of impurities	
Arsenic (as As)	Not more than 3 ppm (0.003%)
Ash (total)	Not more than 5%
Ash (acid-insoluble)	Not more than 2%
Heavy metals (as Pb)	Not more than 40 ppm (0.004%)
Lead	Not more than 10 ppm (0.001%)
Rosin	Passes test

VI. Description

A. General Characteristics

Gum guaiac occurs as irregular masses enclosing fragments of vegetable tissues or larger homogeneous masses. It is brownish black to dusty brown, acquiring a greenish tint on long exposure. The fractured surface has a glassy luster; the thin pieces are transparent and varying in color from brown to yellowish orange. The powder is yellow brown, turning olive-brown on exposure to air. Gum guaiac has a balsamic odor and a slightly acid taste.

B. Physical Properties

Gum guaiac has a melting range of 85 to 90 degrees C. It is insoluble in water, but slightly soluble in benzene and carbon disulfide. Guaiac is freely soluble in alcohol, chloroform, ether, creosote, solution of chloral hydrate, and alkalies. In liquid preparations, gum guaiac is incompatable with mineral acids, acacia, ferric chloride, gold chloride, permanganates, spirit nitrous ether and water.

C. Stability

Store in well-closed containers.

VII. Analytical Methods

The official method for the separation of gums from ice cream and frozen desserts involves treatment with trichloroacetic acid, followed by centrifugation. Addition of saturated sodium chloride solution to the supernatant results in precipitation of the gum. After purification, a gum film is prepared for infrared spectroscopy. The sample spectra must be compared to standard spectra for identification of the gum (4).

Methods for the separation of gums from mayonnaise, salad dressings, meat products, and soft curd cheese are also presented (4).

VIII. Occurrence

A. Plants

Gum guaiac is resin from wood of *Guajacum officinale* L. or *G. sanctum* L., *zygophyllaceae*.

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage mg/kg Body Weight	Measurement	Ref.
Gum Guaiac	Mice	Unk.	i.p.	> 2000	LD_{50}	33
Gum Guaiac	Mice	Unk.	per os	> 2000	LD_{50}	33
Gum Guaiac	Rats	Unk.	per os	> 2000	LD_{50}	33
Gum Guaiac	Rats	Unk.	per os	> 5000	LD_{50}	33
Gum Guaiac	Rats	Both & 10	per os	5000	LD_{50}	25
Gum Guaiac	Guinea pigs	Unk.	per os	1120	LD_{50}	33

Rats

Jenner et al. reported an LD_{50} of 5000 mg/kg for gum guaiac in corn oil. Ten young adult Osborne-Mendel rats were evenly divided by sex and fasted 18 hours prior to intubation of the dose. Toxic signs included rough for contributing to a scrawny appearance. Death occurred within 18 hours (25).

Man

Six human subjects were given 10 doses of 2-3 g gum guaiac at one time. One or two loose stools were passed (27).

II. Short-term STudies

Rats

After feeding young male rats 0.5% gum guaiac for 6 months, Lehman et al. reported the mean growth rate to be 80-85% of the control rat growth rate. A safety factor of 100 is generally taken when recommending levels for antioxidants in fats and shortenings. Since gum guaiac was normally used in concentrations of 0.1%, a 10% level should have been tested. However, technical difficulties prevented such testing. The authors state that the long use of gum guaiac in medicine without any reported injuries appears to substantiate its safety in foods (33).

Cats

Five adult cats were fed daily 0.5 to 1.0 g of gum guaiac (approximately 0.66 g/kg body weight) for 34 to 117 weeks. Three cats received none. Only one cat which received 1 g failed to gain weight. Gross and

histological examination of the lungs, kidneys, livers and spleens revealed no untoward effects. The intestinal mucosa was without irritation or injury as well (27).

Dogs

Johnson et al. examined the effect of guaiacum ingestion in 11 full grown dogs of unspecified sex and size. Five dogs received 0.5 to 1 g daily while another three received 1 g daily (i.e. up to 0.1 g/kg body weight); three dogs served as controls. After 62 to 103 weeks of treatment, all but one dog had gained weight. Red cell and white cell counts were normal, as were hemoglobin determinations. A histological examination of lungs, kidneys, livers, and spleens revealed normal organs. The intestinal mucosa was without irritation or injury as well (27).

Man

Four women and seven men ingested 0.05 or 0.10 g of gum guaiac from 18 to 104 weeks. Five subjects continued for another 90 weeks. The gum was mixed in chocolate pellets. Red and white blood cell counts and hemoglobin determinations were performed monthly. Also, Volhard's urine-concentration kidney function test was made. Stool consistency and body weight were noted. All subjects were healthy with no untoward effects reported (27).

III. Long-term Studies

Lehman et al. cited the following two-year feeding study from Bieter (1949):

	Dosage %	Number of Rats Per Group	Living at 2 yrs.
Gum Guaiac	0	10	7
	0.5%	10	9

No pathologic damage could be attributed to gum guaiac (33).

Four groups of 10 rats were fed a basal diet plus 0, 0.05, 0.5, or 5.0 g gum guaiac per 100 g of lard throughout their lifetime. For three successive generations they ate approximately 0.2 g/kg daily. Growth was normal for all groups in all generations. The average life span for rats of all three generations were comparable for all groups. The kidney, liver, spleen and lungs of 6 control and 17 experimental rats were examined microscopically. No damage could be attributed to gum guaiac (27).

IV. Special Studies

Reproduction

Reproductive virility was determined for 4 groups of 10 rats fed a basal diet plus 0.0, 0.05, 0.5, and 5.0 g of gum guaiac per 100 g of lard. The basal diet included 10% lard. Three successive generations were fed the same levels. There were no significant differences in the number of pregnancies, number of young born, and number of young weaned between the different dietary groups (27).

Biochemical Aspects

I. Breakdown

Gum guaiac is heat stable and withstands the cooking and drying processes used in the preparation of dehydrated materials (38).

II. Absorption - Distribution

Johnson et al. reported that little or none of gum guaiac consumed is absorbed. An average of one half of the gum guaiac fed to dogs appeared in their feces, but the results were quite inconsistant. Only a small fraction of gum guaiac added to feces in vitro could be recovered after incubation at body temperature, in some instances. They concluded that guaiacum must be partially destroyed in the colon. Gum guaiac fed to dogs did not appear in the blood, and when injected intravenously disappeared rapidly from the bloodstream. None was found in urine after feeding. It is important to note that their quantitative test for gum guaiac had an estimated accuracy of 50% (27).

III. Metabolism and Excretion

No Information Available

IV. Effects of Enzymes and Other Biochemical Parameters

Johnson et al. reported that a diet containing 5 g of gum guaiac per 100 g lard resulted in a slight reduction of fat absorption in rats. Levels of 0.05 and 0.5 g/100 g lard had no effect. Dogs receiving 0 or 1 g of gum guaiac daily showed no differences in fat absorption (27).

A slight cathartic action was produced in rats fed 5 g gum guaiac per 100 g lard (27).

V. Drug Interaction

No Information Available

VI. Consumer Exposure Information

Gum guaiac, an antioxidant, may be added directly to foods in amounts up to 0.1%. It is also cleared for use in food packaging in amounts no more than 50 ppm (29).

GUM GUAIAC

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Oxystearin. A specification was developed for oxystearin. As it is manufactured by controlled oxidation of fat, the Committee thought it wise to limit the epoxide content of the material.

Ascorbyl stearate. Specifications were developed for ascorbyl stearate. This substance was evaluated in terms of studies previously reviewed for ascorbyl palmitate in the sixth report,[28] since the latter substance contained an admixture of the stearate. An acceptable daily intake was established for these two substances singly or in combination.

Nordihydroguaiaretic acid. A specification had been developed for NDGA at the third meeting.[29] However, the biological data were inadequate to allow an evaluation.

Tocopherol esters. These were not considered to be effective antioxidants and therefore no specifications were prepared for them.

Isoamyl gallate and ethyl protocatechuate. These substances were not considered as the data were inadequate to proceed to an evaluation and hence no monographs were prepared. A tentative specification for isoamyl gallate and identification tests for ethyl protocatechuate were developed.

Calcium sulfate. A specification was developed for calcium sulfate. No limit was placed on the use of calcium sulfate as a firming agent, except for good manufacturing practice.

Potassium chlorate. This substance was regarded by the Committee to be too toxic to allow its use as a food additive; hence no monograph was prepared for it. Identification tests were drawn up.

Gum guaiac. A specification could not be developed for gum guaiac owing to lack of information and consequently the earlier decision[30] was confirmed. No monograph was prepared for it.

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[28] Annex 1, ref. 6.
[29] Annex 1, ref. 3.
[30] Annex 1, ref. 8.

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Research Section

Food Flavourings and Compounds of Related Structure
I. Acute Oral Toxicity

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Abstract—Oral dosages of 107 synthetic and natural flavourings and structurally-related compounds were administered by intubation to the mouse, rat or guinea-pig. Animals were observed usually for 2 weeks during which time the development of toxic signs was followed and time of death recorded. The acute oral LD_{50} of each compound was determined.

INTRODUCTION

Substances used as food flavourings have received little attention from the toxicological viewpoint. Because of their extensive use as food additives, the Food and Drug Administration has been investigating their toxicity.

The initial step in our toxicity studies was the determination of the acute oral effects. This paper presents data on acute toxicity for a large number of flavouring matters. Similar data are reported for additional compounds, not necessarily flavourings, but included as a means of correlating structure with toxicity. These relationships have been discussed by Taylor, Jenner & Jones (1964) and Hagan, Jenner, Jones & Fitzhugh—*Toxicology;* Long, Brouwer & Webb—*Pathology* (1964).

Flavour additives include compounds with a wide variety of chemical structures, and mixtures of variable composition derived from plants and other natural sources. Some of the substances are synthetic, others are isolates or extracts of natural products. Since the purpose of these studies was to evaluate the toxicity of these materials in relation to their use as food additives, a commercially available material was used. No attempt was made to secure chemically pure compounds.

METHODS

Groups of 10 young adult Osborne–Mendel rats evenly divided by sex were fasted for approximately 18 hr prior to treatment. Groups of guinea-pigs consisting of both males and females were fasted for the same period. Mice were treated on full stomachs. Animals had access to water at all times, and the food was replaced in cages as soon as animals received their respective doses. All doses were given by intubation.

All animals were maintained under close observation for recording toxic signs and time of death. Such observation was continued until animals appeared normal and showed weight gain. The usual observation period was 2 weeks; in a few cases, where no acute toxic signs were seen, the animals were observed for only one week. LD_{50}'s were computed by the method of Litchfield & Wilcoxon (1949).

RESULTS

The LD_{50}'s, slope function, and their confidence limits, together with toxic signs and times of death, are recorded for each compound in Table 1. The species tested, solvent, and solution concentration (w/v) are also listed. Where no solvent is indicated the substance was a liquid and it was administered undiluted.

Table. 1 *Acute oral toxicity of food flavourings and compounds of related structure*

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Acetophenone (methyl-1-phenyl ketone)		Rat	3200 (2460–4160)	1·9 (1·3–2·8)	Coma within 5 min persisting in some rats for 24 hr D.T. 1 hr–4 days
Aldehyde C-10 decyl (decanal)		Rat	>33,320*	—	Excitation, diarrhoea, wet fur on stomach and posterior in both species
		Mouse	>41,750	—	
Aldehyde C-14 (γ-undecalactone)		Rat	18,500 (16,930–20,260)	1·2 (1·0–1·3)	Depression within 10 min. Wet fur D.T. 4 hr–5 days
Aldehyde C-18 prunolide (lactone of 4-hydroxy-nonanoic acid)		Rat	9780 (7480–12,810)	1·6 (1·4–1·8)	Depression, coma D.T. 4–18 hr
do.		Guinea-pig	3440 (2890–4100)	1·5 (1·2–1·8)	Depression, salivation D.T. 4 hr–6 days
Allyl acetate†	A‡—10%	Rat	142 (116–175)	1·6 (1·3–2·0)	Depression soon after treatment. Rough fur, scrawny appearance for several days D.T. 4 hr–6 days
do.	A—2 & 5%	Mouse	170 (152–190)	1·2 (1·1–1·3)	Depression D.T. 4–18 hr
Allylacetic acid† (pentenoic acid)	A—15%	Rat	470 (385–573)	1·5 (1·3–1·8)	Depression within 30 min. High dose caused convulsions. Most rats that recover appear normal the day following treatment D.T. 30 min–18 hr
do.	A—15%	Mouse	610 (460–808)	2·2 (1·5–3·1)	Depression D.T. 1–18 hr
Allyl alcohol†	B—2%	Rat	70 (63–79)	1·6 (1·2–2·0)	Depression, colourless secretion from eyes, diarrhoea, scrawny appearance for several days D.T. 4 hr–4 days

*Highest dose administered

†Toxicity was studied because of structural relation to a flavouring agent.

‡A=corn oil; B=water.

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
p-Allylanisole (1-methoxy-4-allylbenzene)		Rat	1820 (1670–1980)	1·2 (1·1–1·3)	Marked depression, some rats in coma for 24 hr, rough fur, wet posterior, porphyrin-like deposit around eyes D.T. 4 hr–8 days
do.	A—10, 20%	Mouse	1250 (812–1920)	1·6 (0·9–2·8)	Depression soon after treatment, coma on higher doses D.T. 1 hr–4 days
Allylbenzene†		Rat	5540 (4620–6650)	1·5 (1·2–2·0)	Depression, some rats comatose for 2–3 days, wet posterior, scrawny appearance for several days D.T. 4 hr–5 days
Allyl butyrate (allyl butanoate)		Rat	250 (216–290)	1·5 (1·2–1·8)	Depression, wet posterior, scrawny appearance for several days D.T. 4 hr–5 days
Allyl caproate (allyl hexanoate)		Rat	218 (186–255)	1·3 (1·1–1·5)	Depression. Scrawny appearance D.T. 4–18 hr
do.		Guinea-pig	280 (246–319)	1·3 (1·1–1·5)	Depression, salivation D.T. 4 hr–3 days
Allyl cinnamate (allyl 3-phenylacrylate)		Rat	1520 (1290–790)	1·4 (1·1–1·8)	Scrawny appearance D.T. 4 hr–8 days
Allyl cyclohexane propionate (allyl 3-cyclohexyl-propionate)		Rat	585 (480–714)	1·5 (1·0–2·2)	Depression, rough fur D.T. 4–hr 6 days
do.		Guinea-pig	380 (172–834)	1·3 (0·5–3·8)	Depression, salivation, haemorrhage in small intestine D.T. 1 hr–6 days
Allyl formate†	A—5%	Rat	124 (107–144)	1·3 (0·5–3·6)	Depression, scrawny appearance for several days D.T. 4 hr–5 days
do.	A—2%	Mouse	136 (122–151)	1·2 (1·1–1·3)	Depression D.T. 4–18 hr
Allyl heptylate (allyl heptanoate)	A—50%	Rat	500 (392–638)	1·7 (1·2–2·2)	Ataxia D.T. 2–18 hr
do.		Guinea-pig	444 (363–541)	1·6 (1·2–2·3)	Depression D.T. 2–18 hr

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
do.	A—25%	Mouse	630 (514–772)	1·5 (1·2–1·8)	Depression D.T. 2–18 hr
Allyl isothiocyanate	A—10%	Rat	339 (318–361)	1·6 (1·2–2·1)	Scrawny appearance, porphyrin-like deposit around eyes and nose, rough fur D.T. 4 hr–15 days
Amyl acetate (pentyl acetate)		Rat	16,550 (14,370–19,030)	1·2 (1·1–1·4)	Depression, coma, rough fur D.T. 4 hr–2 days
Amyl alcohol (pentanol)		Rat	3030 (1440–6360)	1·2 (1·0–1·4)	Depression D.T. few min–18 hr
Amyl butyrate (pentyl butyrate)		Rat	12,210 (10,260–14,560)	1·6 (1·3–1·8)	Depression, rough fur, wet posterior D.T. few min–2 hr
do.		Guinea-pig	11,950 (8530–16,730)	3·0 (1·2–7·2)	Depression, ataxia D.T. 2 hr–6 days
Amyl cinnamic aldehyde (α-*n*-pentyl-β-phenyl-acrolein)		Rat	3730 (3190–4370)	1·4 (1·2–1·6)	Depression, porphyrin-like deposit around eyes and nose D.T. 4 hr–5 days
Amyl valerianate (pentyl pentanoate)		Rat	>35,420*	—	No effect in 2 weeks observation
do.		Guinea-pig	>17,260*	—	Wet fur D.T. 2–6 days
Amyris oil		Rat	5580 (4540–6860)	1·5 (1·4–1·6)	Ataxia, coma within 1 hr, porphyrin-like deposit around eyes and nose. Wet posterior D.T. 1–3 days
Anethole (1-methoxy-4-propenyl-benzene)		Rat	2090 (1420–3070)	1·8 (1·3–2·4)	Low doses—depression, high doses—coma D.T. 4 hr–4 days
do.		Guinea-pig	2160 (1920–2450)	1·3 (1·2–1·5)	Depression D.T. 1–7 days
do.		Mouse	3050 (2330–4000)	1·6 (1·2–2·1)	Depression, coma within 15 min D.T. 2–4 hr
Anisaldehyde (*p*-methoxybenzaldehyde)		Rat	1510 (1360–1700)	1·2 (1·1–1·3)	Depression D.T. 4–18 hr
do.		Guinea-pig	1260 (937–1700)	1·6 (1·2–2·0)	Depression within 2 hr D.T. 1–3 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Anisole (methoxybenzene)		Rat	3700 (3240–4220)	1·2 (1·1–1·4)	Depression, porphyrin-like deposit around eyes, salivation, bloody urine, rough fur D.T. 4 hr–8 days
β-naphthyl *iso*butyl ether		Rat	5930 (4750–7420)	1·9 (1·4–2·5)	Wet posterior, coma within 1 hr, rough fur and black, soft stools D.T. 1–6 days
Benzaldehyde		Rat	1300 (1110–1540)	1·4 (1·2–1·6)	Depression, coma on higher doses D.T. 4–18 hr
do.		Guinea-pig	1000 (800–1250)	1·4 (1·2–1·8)	Diuresis, tremors, intestinal irritation and haemorrhage D.T. 1 hr–4 days
Benzene†		Rat	4080 (3260–5100)	1·7 (1·4–2·1)	Tremors, loss of equilibrium and comatose on high doses, scrawny appearance for several days D.T. 1 hr–4 days
Benzyl acetate		Rat	2490 (2040–3040)	1·6 (1·1–2·4)	Depression D.T. 4 hr–3 days
Benzyl alcohol		Rat	1230 (1130–1330)	1·2 (1·1–1·3)	Depression, coma within 10–15 min. Excitable for 3–4 days D.T. 1 hr–4 days
do.	A—25%	Mouse	1580 (1410–1770)	1·2 (1·1–1·3)	Depression D.T. 2–18 hr
Benzyl *n*-butyrate (benzyl butanoate)		Rat	2330 (1940–2800)	1·3 (1·1–1·6)	Depression, scrawny appearance, tremors with higher doses D.T. 4 hr–4 days
Benzyl cinnamate (cinnamein, benzyl β-phenylacrylate)		Rat	5530 (3100–7740)	2·2 (0·9–3·6)	Depression, coma persisting in some rats for 24 hr D.T. 4 hr–5 days
do.		Guinea-pig	3760 (2340–6055)	4·2 (1·3–13·8)	Depression, gastro-intestinal tract irritation, rectal bleeding D.T. 4 hr–6 days
n-Butyl alcohol (butanol)		Rat	2510 (2220–2840)	1·4 (1·2–1·7)	Depression, coma D.T. 4–18 hr

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Cajeput oil		Rat	3870 (3360–4450)	1·2 (1·1–1·3)	Scrawny appearance, wet posterior. Gross pathology shows pale, nutmeg livers. Depression, persisting in some animals for as long as 3 days D.T. 4 hr–9 days
Calamus oil		Rat	777 (612–987)	1·7 (1·3–2·3)	Severe tremors from 30 min–2 hr after treatment. Scrawny appearance, weight loss for several days D.T. 4 hr–8 days
n-Caprylic acid (octanoic acid)		Rat	10,080 (8190–12,370)	1·6 (1·3–2·0)	Depression, diarrhoea D.T. 4 hr–9 days
Carvacrol (2-methyl-5-*iso*propylphenol)		Rat	810 (710–920)	1·2 (1·1–1·3)	Depression within 10 min, coma within 1 hr D.T. 1 hr–3 days
Carvone (*p*-mentha-6, 8-dien-2-one)		Rat	1640 (1260–2130)	1·8 (1·3–2·5)	Depression, ataxia (loss of use of hind legs) D.T. 4 hr–5 days
do.		Guinea-pig	766 (603–845)	1·3 (1·1–1·6)	Extreme depression D.T. 4–18 hr
Cedryl acetate		Rat	44,750 (33,650–59,520)	1·6 (1·3–2·0)	Depression, scrawny appearance, rough, wet fur D.T. 4 hr–11 days
Cinnamic aldehyde (cinnamaldehyde)		Rat	2220 (1910–2600)	1·4 (1·2–1·6)	Depression, diarrhoea, scrawny appearance D.T. 2–3 hr
do.		Guinea-pig	1160 (955–1420)	1·5 (1·2–1·9)	Coma with higher doses D.T. 2 hr–4 days
Citraconic acid (*cis*-methylbutenedioic acid)	B—25%	Rat	1320 (1070–1640)	1·6 (1·1–2·4)	Depression, scrawny appearance, tremors, stomach haemorrhage D.T. 4 hr–3 days
do.	B—25%	Mouse	2260 (1930–2640)	1·4 (1·2–1·6)	Depression, laboured respiration, haemorrhage in gastro-intestinal tract D.T. few min–3 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
do.	B—25%	Guinea-pig	1350 (1040–1755)	1·9 (1·1–3·1)	Scrawny appearance, gastro-intestinal tract irritation D.T. few min–3 days
Citral (3,7-dimethyl-2,7-octa-dienal)		Rat	4960 (3940–6240)	1·5 (1·2–2·0)	Depression D.T. 4 hr–4 days
Coumarin§ (2 H-1-benzopyran-2-one)	A—5%	Rat	680 (505–920)	1·8 (1·0–3·5)	Depression soon after treatment, mottled livers D.T. 4 hr–8 days
do.	C‡—10%	Guinea-pig	202 (179–228)	1·2 (1·1–1·4)	Depression, ataxia, severe gastro-intestinal tract irritation D.T. 1–6 days
Cuminaldehyde (*p-iso*propylbenzaldehyde)		Rat	1390 (1140–1700)	1·5 (1·3–1·8)	Depression within 1 hr after treatment, scrawny appearance, porphyrin-like deposit around eyes and nose. Gross pathology showed discoloured liver, irritated gastro-intestinal tract, stomach haemorrhage, yellowish material attached to the intestines D.T. 4 hr–10 days (most deaths within 2 days)
Cyclamen aldehyde (*p-iso*propyl-α-methyl-hydrocinnamaldehyde)		Rat	3810 (3080–4730)	1·7 (1·3–2·2)	Ataxia soon after treatment, coma within 1 hr. Wet fur, porphyrin-like deposit around eyes and nose D.T. 1–6 days
p-Cymene (4-*iso*propyl-1-methyl benzene)		Rat	4750 (3720–6060)	1·7 (1·5–2·0)	Depression soon after dosing, coma, bloody lacrimation, diarrhoea. Irritable, scrawny appearance for as long as 2 weeks D.T. 4 hr–12 days
Diacetyl (2,3-butanedione)		Rat	1580 (1310–1920)	1·5 (1·2–1·9)	Depression followed by convulsions within 10–15 min after treatment D.T. few min–2 hr

‡C=Propylene glycol

§Not acceptable for food use because of toxic effects observed on chronic feeding.

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
do.		Guinea-pig	990 (728–1350)	2·4 (1·2–4·6)	Ataxia, gasping, coma D.T. few min–4 days
Diallylacetic acid†	B—2%	Rat	570 (467–695)	1·6 (1·3–1·9)	Depression soon after treatment, weight loss, scrawny appearance D.T. 3–5 days
Dihydroanethole (1-methoxy-4-propylbenzene)		Rat	4400 (3380–5720)	1·9 (0·6–5·6)	Depression, wet posterior D.T. 4 hr–3 days
do.		Mouse	7300 (5930–9000)	1·6 (1·4–1·8)	Porphyrin-like deposit around eyes and nose, rough fur, scrawny appearance D.T. 1–4 days
Dihydrocoumarin (1,2-benzohydropyrone)		Rat	1460 (1180–1820)	1·6 (1·0–2·6)	Depression D.T. 2 hr–2 days
do.		Guinea-pig	1760 (1460–2170)	1·6 (1·2–2·1)	Depression D.T. 4 hr–4 days
Dihydrosafrole§ (1,2-methylenedioxy-4-propylbenzene)		Rat	2260 (1840–2780)	1·7 (1·4–2·0)	Depression soon after treatment, scrawny appearance, lacrimation D.T. 2–5 days
do.		Mouse	4300 (3420–5420)	1·8 (1·2–2·7)	Severe ataxia, coma D.T. 4 hr–5 days
Dimethylbenzyl carbinol (1,1-dimethyl-2-phenyl-ethanol)		Rat	1280 (934–1770)	2·3 (1·0–5·1)	Depression, coma D.T. 1–24 hr
do.		Guinea-pig	988 (705–1380)	1·7 (1·4–2·0)	Diuresis, coma. Severe gastro-intestinal tract irritation D.T. 1 hr–4 days
Dipropylacetic acid† (2-propylvaleric acid)	B—2%	Rat	670 (598–750)	1·2 (1·0–1·4)	Depression, scrawny appearance, diarrhoea in 1 hr D.T. 2 hr–2 days
Dolcourin‖		Rat	924 (825–1030)	1·2 (1·1–1·3)	Depression, abdominal cavity filled with bloody fluid D.T. 2 hr–5 days
Ethyl butyrate (ethyl butanoate)		Rat	13,050 (12,210–13,940)	1·2 (1·0–1·3)	Depression within a few min, coma on higher doses D.T. 4–18 hr

‖Dolcourin is a trademark name for a substance supplied by Dodge & Olcott, Inc. as a coumarin substitute.

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Ethyl caprylate (ethyl pentanoate)		Rat	25,960 (22,190–30,370)	1·4 (1·2–1·6)	Depression, coma, wet posterior D.T. 4 hr–4 days
Ethyl formate		Rat	1850 (1520–2240)	1·6 (1·3–1·9)	Depression within 5–10 min. Laboured respiration D.T. 15 min–2 hr
do.		Guinea-pig	1110 (887–1390)	2·0 (1·3–3·1)	Depression. Irritated gastro-intestinal tract D.T. 10 min–2 hr
Ethyl methylphenyl glycidate (ethyl ester of 2,3-epoxy-3-methyl-3-phenylproprionate)		Rat	5470 (4670–6410)	1·3 (1·1–1·5)	Depression, rough fur, porphyrin-like deposit around eyes and nose D.T. 4 hr–8 days
do.		Guinea-pig	4050 (3540–4620)	1·5 (1·2–1·8)	Depression in varying degrees, rectal bleeding on high doses D.T. 4 hr–7 days
Ethyl oenanthate (ethyl heptanoate)		Rat	>34,640*		Depression, coma, rough and wet fur
Ethyl oxyhydrate¶		Rat	14,700 (13,300–16,240)	1·1 (0·9–1·4)	Coma within 5 min D.T. 4 hr–5 days
Ethyl pelargonate (ethyl nonanoate)		Rat	>43,000*	—	Depression, irritated gastro-intestinal tract. Appeared normal 24 hr after treatment
do.		Guinea-pig	24,190 (19,350–30,240)	1·5 (1·2–2·0)	Depression, irritated gastro-intestinal tract D.T. 4 hr–6 days
Ethyl sebacate (ethyl decandioate)		Rat	14,470 (12,805–16,350)	1·4 (1·3–1·5)	Wet fur, depression D.T. 4 hr–4 days
do.		Guinea-pig	7280 (5970–8900)	1·3 (1·1–1·4)	Depression, diuresis D.T. 8 hr–6 days
Ethyl vanillin (3-ethoxy-4-hydroxy-benzaldehyde)	C—20%	Rat	>2000**	—	Depression, coma on high doses D.T. 3–18 hr

¶Ethyl oxyhydrate is a mixture whose composition varies with the method of manufacture. The material used in this study was obtained from Florasynth Labs., Inc., N.Y.

**Toxicity of the propylene glycol solvent prohibited administration of higher doses.

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Eucalyptol (1,8-epoxy-*p*-menthane)		Rat	2480 (2100–2930)	1·4 (1·2–1·6)	Depression, coma on high doses, scrawny appearance for 3–4 days. Recovery within 7 days
Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)		Rat	2680 (2420–2970)	1·2 (1·1–1·4)	D.T. 2 hr–4 days Coma soon after treatment D.T. approximately 1 hr
do.		Mouse	3000 (2400–3750)	1·8 (1·3–2·3)	Severe depression immediately after treatment D.T. few min–2 days
do.		Guinea-pig	2130 (1860–2430)	1·3 (1·2–1·5)	Depression D.T. 4 hr–3 days
Eugenol acetate (1-acetoxy-2-methoxy-4-allylbenzene)		Rat	1670 (1265–2200)	1·9 (1·4–2·5)	Rough fur, depression D.T. 4 hr–2 days
Eugenol methyl ether (1,2-dimethoxy-4-allylbenzene)		Rat	1560 (1170–2070)	2·6 (1·6–4·3)	Coma within 1 hr after treatment D.T. less than 8 hr
Fenchone (*d*,1,3,3-trimethyl-2-norcamphanone)		Rat	6160 (4400–8630)	2·7 (1·1–6·5)	Depression, scrawny appearance, porphyrin-like deposit around eyes and nose for a week after treatment D.T. 4 hr–9 days
2-Furaldehyde	A—5%	Rat	127 (110–147)	1·3 (1·2–1·5)	Depression soon after treatment. Scrawny appearance, porphyrin-like deposit around eyes and nose. Gross pathology showed lung haemorrhage on the higher doses D.T. 4 hr–4 days
Geraniol extra (3,7-dimethyl-2,6-octadienol) (3,7-dimethyl-1,6-octadienol)		Rat	3600 (2840–4570)	1·7 (1·3–2·2)	Depression, coma, wet fur D.T. 4–18 hr
Geranyl acetate		Rat	6330 (5450–7340)	1·3 (1·2–1·4)	Depression, coma D.T. 4 hr–3 days
Geranyl butyrate		Rat	10,660 (8020–14,180)	1·6 (0·7–3·7)	Marked depression and coma on higher doses D.T. few min–4 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Guaiac gum	A—20%	Rat	>5000*	—	Rough fur, scrawny appearance D.T. less than 18 hr
Ho leaf oil		Rat	3270 (2780–3830)	1·6 (1·3–2·0)	Coma on higher doses within 30 min. Scrawny appearance, rough fur, porphyrin-like deposit around eyes and nose D.T. 1–4 days
Hydratropic aldehyde (2-phenylpropanal)		Rat	2800 (2390–3280)	1·3 (1·2–1·4)	Coma within 20 min after treatment. Rough fur D.T. 1–5 days
Ionone [60% α-ionone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one and 40% β-ionone, 4-(2,6,6-trimethyl-1-cyclohexen -1-yl)-3-buten-2-one]		Rat	4590 (3880–5400)	1·4 (1·3–1·5)	Depression, tremors D.T. 4 hr–4 days
Isoamyl formate		Rat	9840 (7720–12,550)	1·7 (1·3–2·3)	Depression soon after treatment, scrawny appearance D.T. 4 hr–4 days
Isoeugenol (1-hydroxy-2-methoxy-4-propenylbenzene)		Rat	1560 (1290–1880)	1·4 (1·3–1·5)	Coma soon after treatment, scrawny appearance D.T. 1 hr–7 days
do.		Guinea-pig	1410 (1130–1780)	2·0 (1·5–2·7)	Depression, coma D.T. 3–6 days
Isosafrole§ (1,2-methylenedioxy-4-propenylbenzene)		Rat	1340 (1140–1590)	1·4 (1·2–1·7)	Depression, coma, rough fur, scrawny appearance D.T. 4 hr–8 days
do.	A—25%	Mouse	2470 (2010–3040)	1·7 (1·3–2·2)	Depression soon after treatment. Severe ataxia within 24 hr. Recovery from ataxia but scrawny in appearance within 48 hr D.T. 1 hr–4 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Juniper tar		Rat	8014 (6550–9770)	1·5 (1·2–1·9)	Depression soon after treatment, scrawny appearance for several days. Irritated gastro-intestinal tract D.T. 4 hr–4 days
Linalool (3,7-dimethyl-1,6-octadien-3-ol or 3,7-dimethyl-1,7-octadien-3-ol)		Rat	2790 (2440–3180)	1·3 (1·2–1·4)	Ataxia soon after treatment D.T. 4–18 hr
Linalyl acetate		Rat	14,550 (12,300–17,170)	1·8 (1·3–2·6)	Depression soon after treatment, coma, wet posterior D.T. 4 hr–4 days
do.		Mouse	13,360 (11,920–15,000)	1·2 (1·1–1·3)	Depression within 10–15 min after treatment D.T. 1–3 days
Linalyl cinnamate		Rat	9960 (8230–12,050)	1·4 (1·2–1·7)	Depression. Scrawny appearance, porphyrin-like deposit around eyes and nose, wet fur on posterior 24 hr after treatment D.T. 4 hr–5 days (most deaths in 48 hr)
Linalyl *iso*butyrate		Rat	>36,300*	—	Depression, wet fur, diarrhoea. Appeared normal after 1 week
do.		Mouse	15,100 (12,330–18,500)	1·6 (1·1–2·2)	Depression soon after treatment. Excitable after 1 hr. Rough fur D.T. 4 hr–3 days
Mace oil		Rat	3640 (3170–4190)	1·4 (1·0–2·0)	Depression. Scrawny appearance for several days after treatment D.T. 4 18 hr
Melilotic anhydride (anhydride of *o*-hydroxy-cinnamic acid)		Rat	1510 (1310–1750)	1·3 (1·1–1·6)	Depression D.T. 1 hr–2 days
Menthol (*p*-menthan-3-ol)	A—50%	Rat	3180 (2790–3620)	1·3 (1·1–1·5)	Ataxia, scrawny appearance D.T. 4 hr–3 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Methyl anthranilate (*o*-aminobenzoic acid, methyl ester)		Rat	2910 (2500–3400)	1·4 (0·9–2·0)	Depression, coma D.T. 1–2 days
do.		Mouse	3900 (3260–4680)	1·5 (1·3–1·7)	Depression D.T. 4–18 hr
do.		Guinea-pig	2780 (2210-3500)	1·8 (1·4–2·3)	Depression, gasping, rapid respiration, irritated gastro-intestinal tract D.T. 4 hr–4 days
Methyl benzoate		Rat	1350 (1290–1410)	1·1 (0·7–1·5)	Depression, porphyrin-like deposit around nose, rough fur, wet posterior. Survivors excitable D.T. 2–18 hr
do.	A—50%	Mouse	3330 (2920–3800)	1·2 (1·1–1·4)	Excitation, tremors D.T. few min–18 hr
Methylenedioxybenzene†		Rat	580 (487–690)	1·4 (1·2–1·7)	Depression and coma on high doses, rough fur, porphyrin-like deposit around eyes and nose D.T. 1–6 days
do.	A—25%	Mouse	1220 (976–1520)	1·4 (1·2–1·7)	Depression and coma on high doses, rough fur, porphyrin-like deposit around eyes and nose D.T. 1–5 days
Methyl salicylate (*o*-hydroxybenzoic acid, methyl ester)		Rat	887 (715–1100)	1·5 (1·2–1·8)	Depression soon after treatment D.T. 4–18 hr
do.		Guinea-pig	1060 (873–1300)	1·6 (1·3–1·9)	Convulsions. Irritated gastro-intestinal tract D.T. 1 hr–3 days
Musk ambrette (artificial) (6-*tert*-butyl-3-methyl-2, 4-dinitroanisole)	A—25%	Rat	339 (283–408)	1·4 (1·2–1·7)	Increased respiration and hypersensitivity 24 hr after treatment. Scrawny appearance, wet posterior, rough fur D.T. 1–3 days

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
Nutmeg oil		Rat	2620 (2200–3120)	1·3 (1·2–1·5)	Depression soon after receiving treatment, scrawny appearance for several days D.T. 4–18 hr
Phenylethyl alcohol (2-phenylethanol)		Rat	1790 (1580–2020)	1·2 (1·1–1·3)	Coma within 15 min. Gross pathology shows irritation of the lower half of the stomach on the higher doses D.T. 4–18 hr
Phenylethyl phenylacetate		Rat	15,390 (12,830–18,470)	1·5 (1·4–1·7)	Depression soon after treatment, scrawny appearance for several days D.T. 4 hr–5 days
Piperonal (3,4-methylenedioxy-benzaldehyde)	A—25%	Rat	2700 (2350–3100)	1·5 (1·1–2·0)	Tremors for several hr followed by depression and ataxia D.T. 2 hr–5 days
n-Propanol		Rat	6500 (5800–7280)	1·2 (1·1–1·3)	Coma soon after treatment, scrawny appearance D.T. 2–18 hr
Propenylbenzene†		Rat	3600 (2650–4900)	2·2 (1·3–3·8)	Scrawny appearance, weight loss, wet posterior for 5–7 days after treatment D.T. 4 hr–9 days
n-Propyl acetate		Rat	9370 (7670–11,430)	1·5 (1·1–1·8)	Depression soon after treatment, rough fur, scrawny appearance D.T. 4–18 hr
do.		Mouse	8300 (7280–9460)	1·2 (1·1–1·4)	Depression soon after treatment D.T. few min–18 hr
Propylbenzene†		Rat	6040 (4830–7550)	1·5 (1·2–2·0)	Depression soon after receiving treatment. Scrawny appearance D.T. 1–3 days
n-Propyl *n*-butyrate		Rat	15,000 (12,600–17,850)	1·4 (0·8–2·5)	Depression soon after treatment followed by coma on higher doses. Rough fur, diarrhoea D.T. 1–3 days
n-Propyl formate		Rat	3980 (3350–4740)	1·5 (1·2–1·7)	Depression soon after treatment D.T. 4–18 hr

Table 1 (*Continued*)

Compound	Concentration (w/v) and solvent used	Species	LD_{50} with 95% confidence limits (mg/kg)	Slope function with 95% confidence limits	Toxic signs and death time (D.T.)
do.		Mouse	3400 (3060–3780)	1·7 (1·3–2·2)	Depression soon after treatment D.T. few min–6 hr
Safrole§ (1,2-methylenedioxy-4-allylbenzene)		Rat	1950 (1760–2160)	1·3 (0·6–3·0)	Depression, ataxia, diarrhoea D.T. 1–5 days
do.	A—50%	Mouse	2350 (2010–2750)	1·4 (1·1–1·6)	Ataxia, depression, scrawny appearance D.T. 4 hr–7 days
Terpinyl acetate (*p*-menth-1-en-8-ol ester of acetic acid)		Rat	5075 (4160–6190)	1·5 (1·3–1·7)	Depression, scrawny appearance, porphyrin-like deposits around eyes and nose D.T. 4 hr–5 days
Thymol (*p*-cymen-3-ol,3-hydroxy-*p*-cymene)	C—20%	Rat	980 (817–1180)	1·6 (1·3–2·0)	Depression, ataxia, coma on high doses D.T. 4 hr–5 days
do.	C—20%	Guinea-pig	880 (740–1050)	1·6 (1·2–2·1)	Irritated gastro-intestinal tract, tremors, coma, respiratory failure D.T. 1 hr–10 days
Vanillin (4-hydroxy-3-methoxy-benzaldehyde)	C—20%	Rat	1580 (1390–1810)	1·3 (1·2–1·5)	Coma soon after treatment D.T. 4 hr–4 days
do.	C—20%	Guinea-pig	1400 (1310–1500)	1·1 (1·09–1·2)	Depression within 1 hr D.T. 1–3 days
Veratrole (1,2-dimethoxybenzene)		Rat	1360 (980–1870)	1·6 (1·3–1·9)	Coma within 10 min after treatment. Salivation, porphyrin-like deposit around eyes, diarrhoea, scrawny appearance for 3–4 days D.T. 1–4 days
do.	A—25%	Mouse	2020 (1650–2480)	1·7 (1·4–2·0)	Rapid, laboured respiration; lacrimation; pawing about mouth; hyperactivity followed by coma within 15 min D.T. few min–4 hr

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Condiments et Complexes de Structure Voisine. I. Toxicité Aiguë par Voie Buccale

Résumé—On administra par intubation des doses de complexes faits de 107 condiments synthétiques et naturels, de structure chimique voisine, à des souris, des rats et des cobayes. On observa habituellement les animaux pendant 2 semaines, durant lesquelles on suivit le développement de signes toxiques et on nota la date de la mort. Pour chaque complexe on détermina la dose orale limite au-delà de laquelle commence l'intoxication aiguë.

Lebensmittelgeschmackszusatze und Verbindungen verwandter Struckturen I. Akute Oraltoxititat

Zusammenfassung—107 synthetischen un natürliche Geschmackszusätze und strukturverwandte Verbingdungen wurden durch Intubation an Mäuse, Ratten und Meerschweinchen verabreicht. Die Tiere wurden gewöhnlich 2 Wochen lang unter Beobachtung gehalten, während welcher Zeit die Entwicklung toxischer Symptome verfolgt und die Zeit des Todeseintritts registriert wurde. Die akute orale mittlere tödliche Dosis jeder Verbindung wurde festgestellt.

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ACTION OF GUM GUAIACUM UPON THE ANIMAL ORGANISM [1]

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The resinous gum of the tropical guaiacum tree has been known to medicine for centuries and has been used extensively in the treatment of various ailments, especially syphilis—Freind (1927) and Wootton (1910)—for which it was considered a specific. Quincy (1749) describes its use in gout, dropsies, "cutaneous foulnesses," catarrhs, ulcerations, gleets, and gonorrhea. The gum was believed to be a "sweetener and cleanser of the blood," a diaphoretic, and was used for "cleansing the joints" and "warming and strengthening the nerves," according to Quincy.

The claims made for the beneficial actions of guaiacum were of course exaggerated, and the substance has no place in the armamentarium of modern medicine though even yet Webster's dictionary (1932) defines the resin as being "used medicinally as a remedy for gout, rheumatism, and skin diseases."

Recent experimental work has shown, however, that guaiacum may come to occupy a useful place of importance economically and to public health. Grettie (1933) has shown that the addition of very small quantities of the gum to lard (.05 gm. in 100 gm. of lard) greatly retards the development of oxidative rancidity. Evans (1932), in a review on vitamin E, emphasizes the destructive action of rancidity in fats upon vitamin E, stating that "so easily is vitamin E destroyed by slight changes in the rancidity of the fats in which it is carried in solution that any analysis of vitamin E without simultaneous analysis for anti-oxygenic activity renders vitamin E analysis worthless." These findings led Johnson, Carlson, and Bergstrom (1938) to test whether the addition of guaiacum to fat-containing diets, which were then treated to induce rancidity, would enhance reproduction by delaying the development of rancidity. They found reproduction to be greater when the diets of rats were protected against development of rancidity by guaiacum.

IS GUAIACUM NONTOXIC?

Taking advantage of this anti-oxidant action of guaiacum would mean its widespread use in foods. Before this can be done safely,

[1] Aided by a grant from Swift and Company.

however, it is necessary to establish beyond all reasonable doubt that the material is nontoxic. The extensive experiments reported here were directed toward answering this question: Is guaiacum nontoxic and therefore safe to use in food products, such as lard, which otherwise readily undergo spoilage from oxidative rancidity with consequent possible destruction of vitamin E? Of course, the extensive use of guaiacum in medicine in the past constitutes some evidence of nontoxicity, for great quantities of the material must have been ingested, apparently with impunity. However, more accurate and controlled data upon this subject was desirable.

The first possible point of action of guaiacum taken by mouth would be upon the gut itself—upon its digestive functions, its motility, its gross and histological appearance. Experiments aimed at discovering any such effects were carried out upon several species of mammals. Next we sought to find out the fate of guaiacum which is ingested. How long does it remain in the gut? Is it destroyed here? Does any of it get into the blood stream? These studies were largely chemical in nature and must be interpreted with caution, because the method used for quantitative detection of guaiacum was not entirely satisfactory. Furthermore, it is conceivable that though guaiacum itself may not be absorbed, some injurious component of it, which fails to give the characteristic chemical reaction for guaiacum itself, may be.

More emphasis was placed, therefore, upon the biological tests. Extensive experiments were conducted to test whether the animal organism as a whole is injured in any way by guaiacum ingestion whether it be from the guaiacum itself or from any conceivable fraction or disintegration product. The maintenance of weight of adults, the growth of young animals, the nutritive value of lard to which guaiacum has been added, the blood picture, reproductive virility, longevity, and kidney function were investigated. This was done chiefly upon rats, dogs, and cats. Finally, as many of these indices of well-being as possible and the influence of guaiacum ingestion upon them, were studied upon human subjects.

It should be emphasized that in all the work extremely large quantities of guaiacum were fed, as well as quantities which were adequate to prevent rancidity. In using these pharmacological doses it was hoped that any possible slight untoward effects might be brought to light.

ACTION OF GUAIACUM IN THE GUT

Digestion and Utilization of Fats Containing Guaiacum: In vitro experiments were performed in which lard containing varying quantities of the anti-oxidant were digested with a bile salt-pancreatic

lipase mixture. Preliminary experiments by Davis (1936) led to the adoption of this procedure. To 30 grams of lard in a 125-c.c. Erlenmeyer flask 20 c.c. of .5 per cent bile salt solution was added. This was incubated at 38°C.(100.4°F.) for 20 minutes in an electric shaker. To the emulsion .5 gram of Wilson's lipase was added. This was rotated in the incubator at 38°C. for either 6 or 12 hours. In all experiments samples of lard containing varying quantities of guaiacum were run simultaneously with a control. After hydrolysis, the material was extracted with benzene and titrated for its fatty-acid content. From the results the percentage of fat digested could readily be computed. It should be noted that though the tests were carried out *in vitro* the attempt was made to simulate body condi-

TABLE 1

In Vitro Digestion of Lard With Varying Quantities of Guaiacum Incubated With Bile Salts and Pancreatic Lipase for Periods of 6 and 12 Hours[1]

Incubation period	6 hours	12 hours
Guaiacum in lard tested	Digested	Digested
pct.	*pct.*	*pct.*
0.000	17.59 ± .47	22.46 ± .18
0.025	17.34 ± .46	22.36 ± .18
0.250	17.12 ± .51	22.31 ± .17
2.500	17.30 ± .49	22.44 ± .22

[1] Each figure is the average (with probable error) of 15 analyses.

tions as regards temperature, fat emulsification, and gut motility as well as reaction and enzyme action. The results (Table 1) analyzed statistically indicate that adding up to 50 times as much guaiacum as is necessary to prevent oxidative rancidity does not interfere with such *in vitro* hydrolysis. Even the controls do not show complete hydrolysis, of course, because of well-known reversibility of the reaction in which fats are hydrolyzed, according to Dietz (1907). An equilibrium is soon reached in which esterification proceeds as rapidly as hydrolysis.

Other experiments were aimed at getting indirect evidence of digestibility *in vivo*. Four groups of 10 rats each were fed identical diets. To the fat of the diet, varying quantities of guaiacum were added as follows: Diet 1, none; Diet 2, .05 gm.; Diet 3, .5 gm.; Diet 4, 5 gm. per 100 grams of lard, which constituted one-tenth of the diet by weight. Analyses by the method of Saxon (1914), slightly modified, were then made of the fat content of the feces as a measure of digestibility. The fat which passed into the feces

(unused fat) would presumably be that proportion which failed to be digested and absorbed.[1]

Analyses showed that the following percentages of fat fed were not used and appeared in the feces:

Diet 1—No guaiacum (controls)—.65 ± .07%;
Diet 2—.05 gm. guaiacum per 100 gm. lard— .67 ± .06%;
Diet 3— .5 gm. guaiacum per 100 gm. lard— .88 ± .06%;
Diet 4— 5 gm. guaiacum per 100 gm. lard—1.26 ± .08%.

These figures are based upon eight duplicate analyses of three- or six-day collections of feces in each group. Only in the rats of Diet 4 is there a statistically significant increase in fat excretion. The lard in the diet of these rats, however, contained 100 times as much guaiacum as is necessary to prevent oxidative rancidity. The increase was probably of little or no physiological significance to the rats of this group, as indicated by observations on the growth of these animals, which will be discussed later.

Similar observations were made upon dogs. Four dogs were fed daily for 12 weeks on a diet containing 48 grams of fat. One week the dogs received no guaiacum and the next week they received one gram of guaiacum daily, mixed with and partially dissolved in the fat of the diet. During four days of each week the feces were collected and analyzed for fat. Since the quantity of fat ingested over this period was known, the quantity not utilized could be calculated. During control weeks (48 duplicate analyses) the dogs excreted 3.73 ± .17 per cent of the fat they ate. During weeks of guaiacum feeding (36 duplicate analyses) the dogs excreted 4.92 ± .17 per cent of the fat fed. These differences are only on the border line of statistical significance despite the fact that the animals were receiving 40 times as much guaiacum per 100 grams of fat as is necessary to prevent oxidative rancidity of lard.

Water Content of Feces: The 40 rats, whose fecal excretion was described above, were studied for a possible cathartic action of guaiacum. This inert material might stimulate gastro-intestinal motility or might mechanically or chemically irritate the mucosa. In either case, one should expect an effect upon the consistency of the stools. These were therefore analyzed for water content. The result showed the following percentages of moisture in the stools, based upon eight

[1] The significance of these experiments is not affected by the recent work of Krakower (1934) which indicates that a large percentage of the fecal fat in human beings represents not unabsorbed fat but fat excreted into the intestine. Krakower's work seems to show that a relatively constant amount of fat in any individual is excreted daily. Granting this, any significant change in the digestion and absorption of fat from guaiacum ingestion should still change the total quantity appearing in the feces.

duplicate analyses of three- or six-day collections of feces in each group:

Diet 1—No guaiacum (controls)—24.4 ± 1.3%;
Diet 2—.05 gm. guaiacum per 100 gm. lard—22.3 ± .68%;
Diet 3— .5 gm. guaiacum per 100 gm. lard—27.2 ± .66%;
Diet 4— 5 gm. guaiacum per 100 gm. lard—34.4 ± .89%.

The figure for the rats of Diet 4 is significantly greater than that for the controls, indicating that in these high concentrations (100 times that necessary to prevent rancidity) some cathartic action is produced on rats. No significant effect in this direction was observed in the rats on Diet 3, however, even though the lard in the diet of these animals contained ten times as much guaiacum as is necessary to prevent rancidity.

Fecal water content was also studied on seven dogs. Two controls received no guaiacum, three received .5 gram daily, and two received 1 gram daily. Otherwise, the diets were the same. In each group, more than 20 duplicate analyses of 24- or 48-hour fecal samples were made. The results, expressed in percentage of dry matter in the feces, are as follows:

Dogs fed no guaiacum—38.2 ± .61%;
Dogs fed .5 gm. guaiacum per day—41.5 ± .92%;
Dogs fed 1 gm. guaiacum per day—41.0 ± .80%.

The figures, statistically not significant, show that the ingestion of as much as a gram of guaiacum per day had no influence upon the water content of the stools of dogs.

Gross and Microscopic Appearance of Gastro-Intestinal Mucosa: The gastric and intestinal mucosa of all rats, cats, and dogs used in these experiments were examined at autopsy, both grossly and in some cases histologically, and in no instance was there any hyperemia, ulceration, or any other evidence of chronic irritation.

FATE OF GUAIACUM IN THE ORGANISM

Recovery of Guaiacum Fed: The work on this subject is based upon a quantitative test for guaiacum that is not so accurate as one would desire. The test depends upon the development of a blue coloration when guaiacum plus blood plus H_2O_2 are brought together. The dilution at which the color persists for at least one minute is used in the quantitative estimation and is only accurate within about 50 per cent.

In a group of six dogs varying quantities of solid guaiacum were fed (without food), and after periods of from 10 to 24 hours the animals were killed; the feces and the contents of the stomach, duo-

denum, small intestine, and large intestine were analyzed for guaiacum. In this short series 83 per cent of the material fed was recovered, distributed as follows: from the stomach, 58.5 per cent of the amount fed; from the duodenum, .3 per cent; from the small intestine, 6 per cent; from the large intestine, 12.5 per cent; from the feces passed, 6 per cent. This tends to show that the material may remain in the stomach for a long time, and that probably little if any is absorbed.

Experiments more physiological in nature were done in which single large doses of guaiacum were mixed with food and fed to dogs. On successive days thereafter feces were collected and analyzed in an attempt to determine how much of the guaiacum passed through the gastro-intestinal tract and how soon it did so. In four dogs fed 20 or 40 grams and one fed two grams it took from two to four days for the feces to become guaiacum-free. Here again we encounter the slow passage of the material through the alimentary canal. The amounts recovered in the feces ranged from 67 to 99 per cent of that which was fed.

Also, feces were collected on four dogs fed .5 gram or 1 gram of guaiacum daily for periods varying from nine to 18 days. It was found here that the feces were guaiacum-free on many days, sometimes for two or three successive days, and that relatively large amounts would be excreted on some days. Quantitative estimates of the total amount of guaiacum recovered in all these experiments were not very consistent, but the results indicated that on the average approximately one-half of the amount ingested does not appear in the feces.

Fate of Guaiacum Not Recovered: What happens to that guaiacum which fails to appear in the feces? Is it destroyed by the digestive juices? Does it disintegrate in the colon, mixed with feces? To test the former possibility guaiacum has been subjected to the action of the digestive juices and of feces *in vitro*. In 22 experiments one-half gram, one gram, or two grams of guaiacum were exposed to the action of dog gastric juice for four, five, or eight hours in a shaker kept at 38°C.(100.4°F.). In every experiment all the guaiacum, within the limits of accuracy of the test, was recovered in the residue. None of it went into solution, and none of it was destroyed. One would expect this result in view of the fact that guaiacum is not soluble in acid solutions.

Upon exposure to artificial pancreatic juice in the same manner (14 experiments) little if any of the material went into solution or was destroyed by the juice. Upon including bile in the mixture

approximately a tenth of the guaiacum went into solution, but there was no definite indication of destruction of the material.

On the other hand, appreciable quantities of guaiacum are apparently destroyed in the colon. At any rate, after adding known quantities of guaiacum to feces *in vitro,* it was possible to recover only a small fraction of the guaiacum after incubation at body temperature for 24 hours, in some instances.

The possibility remains, nevertheless, that some of the material is absorbed into the blood stream. We have found that normal blood and urine give negative tests for guaiacum by our method and that after addition of guaiacum *in vitro* these fluids give positive tests. Twelve dogs were fed two to four grams of guaiacum at one time and were killed at intervals of from four to 17 hours. The whole blood was examined for guaiacum. In 11 instances none was found by our tests; in one instance 7.5 mgm. were found in 500 c.c. of blood. When guaiacum is injected directly into the blood, it rapidly disappears; in seven dogs 9 mgm. was the most guaiacum found five hours after intravenous injection of 300 to 400 mgm.

Body fat taken from subcutaneous and mesenteric deposits of 11 dogs and eight cats fed guaiacum many months gave negative tests for guaiacum in every instance.

In no instance (12 tests) did the feeding of guaiacum (two to four grams) to dogs lead to a positive test for the material in the urine. When the substance is directly injected into the blood stream, however, the urine becomes positive for guaiacum in some instances. In 17 of a total of 19 intravenous injections (of 300 to 600 mgm.), from 0 to 10 per cent of the quantity injected was recovered from the urine. In two instances 30 and 100 per cent were recovered.

The obvious objection to these blood studies is that inasmuch as guaiacum is a mixture and our test may indicate the presence only of one component, we have no evidence about the absorption of other components, which may be toxic. Therefore, tests were made of the effects of intravenous injections in unanaesthetized dogs. A total of 31 injections of .2 to .8 gram each were made upon six dogs. No acute or delayed untoward effects were obtained, except for alcoholic intoxication owing to the alcohol used as a solvent for guaiacum. Control animals receiving alcohol alone, intravenously of course, displayed the same symptoms.

We see from this series of experiments that much of the guaiacum fed passes out in the feces, that an appreciable quantity may be destroyed in the colon, that little or none of it is absorbed, and that even if it should be absorbed it apparently is nontoxic. Attention is again called to the very large doses employed in these experiments.

It was felt of course that these acute experiments were not adequate in themselves. Prolonged experiments were necessary in which as many criteria of general physiological well-being as possible should be used without relying solely upon actual chemical tests. These experiments are described below.

EFFECT OF GUAIACUM UPON BODY WEIGHT, BLOOD PICTURE, AND GROWTH

Weight Maintenance of Dogs and Cats, and Blood Counts Upon Dogs: The effect of guaiacum ingestion upon body-weight maintenance was studied in 11 full-grown dogs fed a standard diet consisting of a mixture of beef lung, 300 grams; white bread, 300 grams; yeast, 8 grams; bone meal, 45 grams; and cod liver oil, 1 teaspoonful daily, for 62 to 103 weeks. Three control animals received no guaiacum, five dogs got from .5 to 1 gram daily, and three got 1 gram daily throughout. Every dog except one not only maintained his weight but actually gained. The one exception was a dog weighing 19 kilograms which was fed one gram of guaiacum daily. This dog lost two kilograms in 75 weeks, which seems insignificant. The general behavior and appearance of all dogs were quite normal in all respects. Red-cell counts, white-cell counts, and hemoglobin determinations made three times upon each of the 11 dogs at intervals during the experiment failed to reveal any deviations from normal in any case.

A similar experiment was conducted upon eight full-grown cats for 34 to 117 weeks. The cats received the same diet as the above-mentioned dogs. Three received no guaiacum and five were fed .5 to 1 gram of guaiacum daily. Only one cat in the group receiving 1 gram of guaiacum daily failed to gain weight. None was apparently adversely affected by the large quantities of guaiacum ingested.

The dogs received daily amounts of guaiacum up to .1 gram per kilogram of weight, to receive which a man weighing 60 kilograms would have to eat 12,000 grams of lard (containing .05 per cent guaiacum) daily. The dosage fed the cats was even higher, approximating .66 gram per kilogram.

Nutritive Value of Lard Containing Guaiacum: In the above-described experiments on body weight of cats and dogs, as well as in the growth experiments on rats to be described in the next section, the objection might be raised that the different animals, fed *ad libitum*, may have eaten different quantities of food and that, therefore, any deficiency in the nutritive value of lard containing guaiacum might be masked by the guaiacum-fed animals simply eating more food. The following experiment was devised to test more rigorously the

relative nutritive value of lard containing guaiacum as compared with lard free from guaiacum.

Forty rats were divided into four groups and fed the following basal diet: casein, 18 per cent; starch, 48 per cent; yeast, 5 per cent; wheat germ, 3 per cent; inorganic salts, 4 per cent; agar, 2 per cent; lard, 10 per cent; butter, 10 per cent. Group 1 received

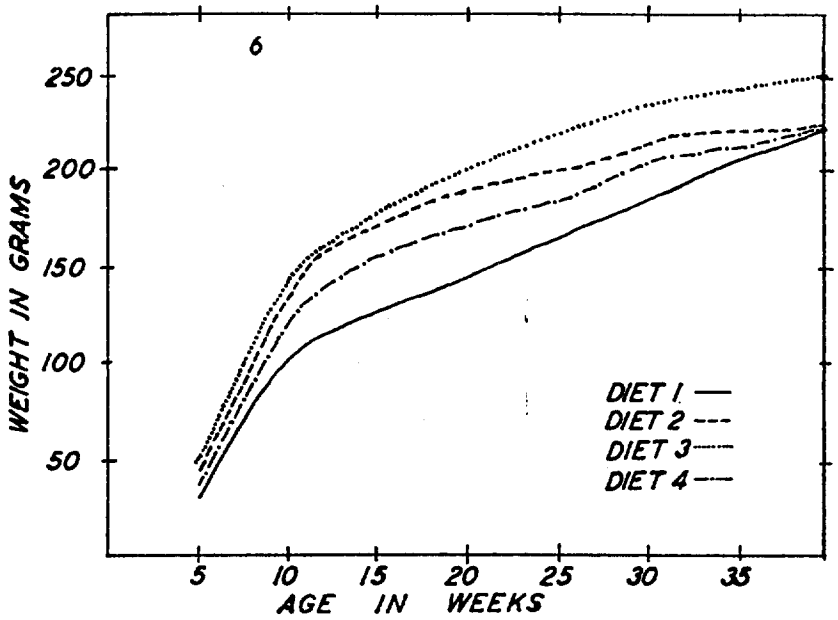

FIG. 1. Growth curves of four groups of ten rats each. The rats in each group were fed the same number of grams of the same basal diet per kilogram of rat per day. The nutritive value of the basal diet plus guaiacum was thus compared with that of the basal diet alone.

nothing else; Group 2 received .05 gram of guaiacum per 100 grams of lard; Group 3, .5 gram of guaiacum per 100 grams of lard; and Group 4, 5 grams per 100 grams of lard. Diet 4 thus contained 100 times as much guaiacum per 100 grams of lard as is needed to prevent rancidity. The rats were fed this for 41 weeks. The feeding was done by the method of Still and Koch (1928), which insures a minimum of scattering of food as well as the ingestion by each group of rats of the same amount of food per gram of body weight per day. Thus no variation in rate of growth can be attributed to one group eating more food than another. This method also provides for the rats getting a little less food than the optimum for a maximum rate of growth. Any deficiency in any diet would thus show up. The growth curves are plotted (Fig. 1); if there is any significant difference here, it is in favor of the groups getting guaiacum. At

least the indication is that lard containing guaiacum is quite as adequate in contributing to rat growth as is lard without guaiacum. In this experiment, of course, the results indicate that the digestion, absorption, and utilization of proteins and carbohydrates as well as fats, is not interfered with.

Growth in Three Generations of Rats: In this experiment rats were submitted to an extremely rigid test of possible toxicity of guaiacum by addition of large quantities of the gum to an otherwise adequate diet and extending the observations over three generations. Forty rats were divided into four groups and fed the same basal diet as that of the rats in the preceding experiment. Then, in addition, the following quantities of guaiacum were fed, mixed with and dissolved in the lard of the basal diet:

Diet 1—None (control group);
Diet 2—.05 gm. per 100 gm. of lard;
Diet 3— .5 gm. per 100 gm. of lard;
Diet 4— 5 gm. per 100 gm. of lard.

Diet 2 contained just the quantity of guaiacum necessary to prevent oxidative rancidity. Diet 4 contained 100 times as much guaiacum as this. Throughout the lifetime of these rats and for three successive generations they ate approximately .2 gram of guaiacum daily per kilogram of body weight. A 60-kilogram man would have to eat 24 kilograms of guaiacum-treated lard per day to ingest this much guaiacum. This is over 400 times the per capita consumption of lard, shortening, and margarine in the United States.[1] The feeding of these tremendous quantities of guaiacum should reveal the presence of even mild toxicological effects.

The second and third generation descendants (80 in number) of the original rats were maintained throughout their lifetime on the same diet as their parents. The usual care was taken with regard to cleanliness of rats, cages, and bedding. The growth curves of the rats of all three generations are shown (Fig. 2) over the normal growth period of 40 weeks. The curves for rats on all four diets lie remarkably close together. At no time does any one curve differ from any other by more than 25 grams.

For comparison, Fig. 2 includes the growth curves of normal rats, as given by Donaldson (1924). This curve lies below all the curves for our rats, indicating clearly that normal growth occurred in the rats on all of our diets.

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[1] According to Swift and Company's data the average per capita consumption of these fats is 55 grams per day.

REPRODUCTION IN THREE GENERATIONS OF RATS

Reproductive virility was determined for the rats used in the preceding experiment by observations upon the number of pregnancies, number of young born, and number of young weaned (Table 2). The time unit in terms of which pregnancies, etc., are expressed is called the "adult female rat weeks." The number of adult females observed multiplied by the number of weeks they were kept with adult males on the same diet equals the number of such rat weeks; for example, 100 rat weeks would mean 10 female rats

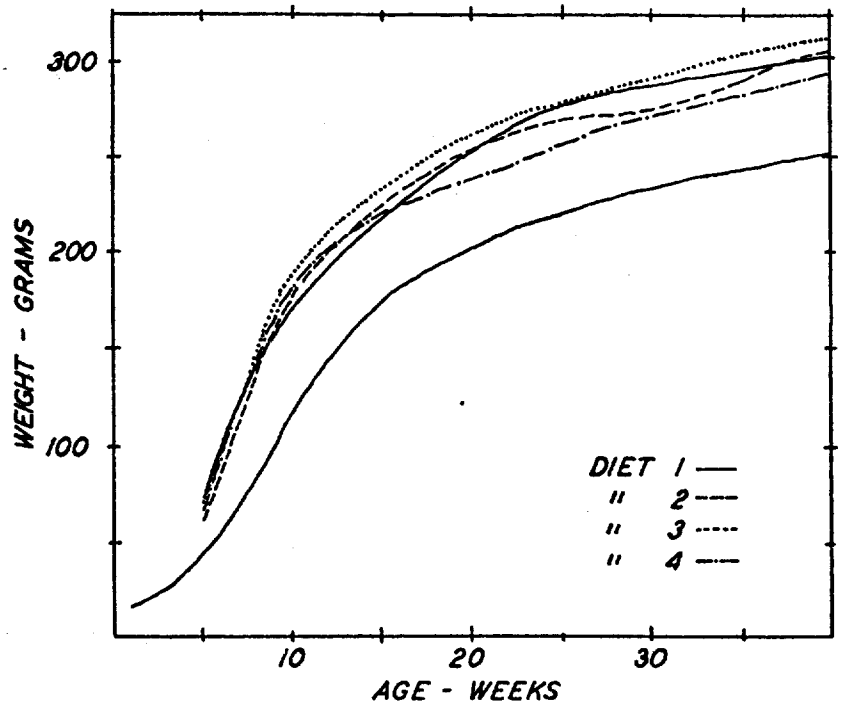

FIG. 2. Growth curves of 120 rats of all three generations. The lower curve (solid line) is based upon data given by Donaldson (1924).

observed for 10 weeks, or 12 female rats observed for 8 plus weeks. Special attention should be paid the third, fifth, and seventh columns (Table 2). Column 3 is a comparison of the average interval elapsing between successive pregnancies. It is an expression of the rate at which pregnancies occurred on the various diets and in successive generations. In this regard, the performance of rats on all three guaiacum-containing diets was about equal to, or better than, that of rats on the control diet, even if the results on the third generation of the control diet, which were poor, are disregarded.

The average number of young born per pregnancy (Column 5) is slightly less in the Diet-4 rats (most guaiacum) than in the control group. The performance of rats on Diet 3, however, equaled that of the control group, though Diet 3 contained 10 times as much guaiacum per 100 grams of lard as is necessary to prevent oxidative rancidity.

TABLE 2

Fecundity of Three Generations of Rats on Diets Containing Varying Quantities of Guaiacum

Column		1	2	3	4	5	6	7
Diet	Generation	Total adult female rat weeks	Total number pregnancies	Average interval between pregnancies (weeks)	Total young born	Young per pregnancy	Total young weaned (21 days)	Per cent weaned
1	1	226	29	7.8	192	6.6	157	82
	2	211	18	11.7	146	8.1	139	95
	3	103	6	17.2	52	8.7	31	60
	1-3	540	53	10.2	390	7.4	327	84
2	1	218	25	8.7	165	6.6	144	87
	2	219	24	9.1	168	7.0	145	86
	3	218	28	7.8	172	6.1	120	70
	1-3	655	77	8.5	505	6.6	409	81
3	1	223	27	8.4	202	7.5	179	89
	2	215	31	6.9	233	7.5	207	89
	3	212	18	11.8	137	7.6	112	82
	1-3	653	76	8.6	572	7.5	498	87
4	1	224	23	9.7	155	6.7	126	81
	2	216	21	10.3	135	6.4	115	85
	3	196	27	7.3	158	5.9	123	78
	1-3	636	71	9.0	448	6.3	364	81
Grand total		2,484	277	9.0	1,915	6.9	1,598	83
Published norms		.....	.....	11.8 [1]	.....	7.0 [1]	.....	86 [1] 78 [2]

[1] Donaldson (1924). [2] Evans and Bishop (1923).

Column 7 of the table shows only very slight, insignificant differences in the percentage of new-born rats which survived the period of weaning.

The last line of the table is given to show that the fecundity of all the rats used in the experiment compares favorably with published data on large series of normal rats as regards frequency of pregnancies,[1] number of young per litter, and proportion of young

[1] The figure, 11.8, for the average interval between pregnancies, is not strictly comparable to ours, of 9 weeks. It is computed from the following data from Donaldson: "Reproductive period in the rat equals 65 weeks; average number of litters during this period, per rat, equals 5.5."

born which were successfully weaned. Also, the average number of pregnancies per rat was identical, at 5.5, in our series and as given by Donaldson (1924).

By these tests of reproductive activity, Diet-4 rats (most guaiacum) compare quite favorably with the controls. The rats on Diet 3 are, if anything, slightly superior to the control rats. The litters of this group were dropped more frequently, and a slightly larger percentage of the young were weaned.

The data indicate that guaiacum produces no deleterious effects upon reproduction when fed in large quantities over a period of three generations of rats.

LONGEVITY

Including all animals the average life spans for rats of all three generations were as follows: Diet 1 (controls), 62.4 ± 3.9 weeks; Diet 2, 79 ± 4.6 weeks; Diet 3, 77.5 ± 5 weeks; Diet 4 (most guaiacum), 72.8 ± 4.5 weeks. The differences in these figures are not statistically significant. When those rats which died within the first year are excluded, average longevity figures are as follows: Diet 1 (controls), 89 weeks; Diet 2, 100 weeks; Diet 3, 100 weeks; Diet 4 (most guaiacum), 93 weeks. According to Donaldson, these rat ages would correspond to ages in man of approximately 53, 60, 60, and 56 years, respectively.

The four individual rats in the entire series which lived longest included one representative from each diet as follows: Diet 1 (control), 147 weeks (equals 88 years in man); Diet 2, 145 weeks (87 years); Diet 3, 141 weeks (85 years); Diet 4 (most guaiacum), 143 weeks (86 years).

These data may be compared favorably with figures given by Donaldson, who states that rats rarely live 170 weeks and that they tend to become weak and decrepit at 100 weeks.

Again, there is no apparent effect upon the life span from feeding large quantities of guaiacum.

MICROSCOPIC FINDINGS

Microscopic sections were made of the kidney, liver, spleen, and lungs of six control rats and 17 rats on the guaiacum diets. In no tissue was there evidence of chronic damage. Particular attention was paid to the liver and kidney, where chronic intoxication might be expected to leave histological evidence. None was found. Two of the guaiacum-fed rats showed slight glomerular damage to a degree judged insufficient to produce impaired function. In any case, the damage could not be ascribed to guaiacum, because most of the

rats fed guaiacum were free from such change, and also, the same mild lesion was observed in one of the controls.

A number of the rats showed small areas of focal necrosis of cells in the liver. This condition, of unknown etiology, was present in the livers of all the controls examined, so that it cannot be ascribed to guaiacum ingestion.

Most of the animals showed changes in the lungs, indicating that pulmonary infection was the cause of death; edema, hemorrhage, bronchitis, broncho-pneumonia, and even gangrene were common. The second most common immediate cause of death was an ailment of undetermined etiology associated with a terminal diarrhea, affecting control and guaiacum rats alike. There were no consistent histological changes in these animals.

Careful examination of the intestine for evidences of irritation of the mucosa by guaiacum was considered to be important. The gastro-intestinal mucosa is the only tissue with which we can be certain ingested guaiacum comes into contact. The histological preparations of rat intestine were unsatisfactory for this study because most of the rats, allowed to die eventually from "natural" causes, could not be autopsied immediately after death. Consequently, post-mortem autolysis of the intestinal mucosa was extensive. Therefore, histological findings obtained on dogs and cats were considered especially significant. Histological sections from three dogs (fed 1 gram of guaiacum daily for 75 weeks) and from two cats (fed .5 to 1 gram of guaiacum daily for 74 weeks), which were killed and autopsied at once, showed a perfectly normal intestinal mucosa in each case, with no suggestion of irritation or injury.

Gross and histological examination of the lungs, kidneys, livers, and spleens from these dogs and cats also revealed normal organs.

EXPERIMENTS ON HUMAN SUBJECTS

Effect of Large Single Doses of Guaiacum: It was considered desirable to extend as many of these observations as possible to human subjects. In all, six human subjects took a total of ten doses of two or three grams of guaiacum at one time. Three grams of guaiacum are equivalent to the amount found in 6,000 grams of guaiacum-lard. On the basis of the per capita daily lard consumption of about 30 grams, this is a six-months' supply. The only untoward action from these large quantities was that one or two loose stools were passed after the ingestion in some instances.

Effect of Daily Ingestion for Two Years: Eleven graduate students and staff members (four women and seven men) ingested .05 or .10 gram of guaiacum daily for periods of 18 to 104 weeks. Five

subjects continued the experiment over 90 weeks. The subjects were on a normal, adequate diet for the whole period. The guaiacum ingested was mixed into little pellets of chocolate. The amounts taken daily are equivalent to that which would be ingested in 100 or 200 grams of lard containing guaiacum in a concentration of .05 gram per 100 grams of lard. These amounts were chosen so as to be well in excess of the per capita daily consumption of lard. Lusk (1928) quotes data of Voit and Rubner on the total fat content of normal dietaries. These figures vary from 46 grams per day for a man weighing 70 kilograms doing light work to 100 grams for a man doing heavy work. Lusk also cites data from Rubner, giving 31 to 65 grams as the per capita daily total fat consumption in four European cities, based on municipal statistics. Inasmuch as these figures are for total fat ingested, we may be sure that the consumption of lard and all lard products would be well under this figure.

Red and white blood-cell counts and blood-hemoglobin determinations were made monthly. Also, each month Fishberg's (1930) modification of Volhard's urine-concentration test for kidney function was performed on each subject. Observations were also made of body weight, number and consistency of stools, general physical condition, and subjective effects.

The red blood-cell counts are plotted, giving the highest and lowest count observed each month in any subject as well as the average for the group of 11 subjects (Fig. 3). The hemoglobin determinations all fell within the normal range of 80 to 100 per cent except for two observations, one of which was 70 per cent, another 75 per cent.

The white blood-cell counts show the range within which all counts fell as well as the average for the group (Fig. 3). It is clear that both the red and white counts fall within the normal range throughout the experiment and that no significant trend in either direction occurred.

None of the subjects either lost or gained significant amounts during the experiment.

Results of studies on the effect of guaiacum ingestion upon number of bowel movements give the average number of stools per week for each month of the experiment, including the range as well as the average for the group (Fig. 4). The curves show no significant trend throughout the period of observation.

That the figures throughout are within the normal limits is indicated by comparison with control data derived from a dietary experiment performed in our laboratory several years ago. At that time it was found that for a ten-week period, 14 subjects passed

an average of 10.5 stools per week, as compared with an average of 9.5 stools in the 11 subjects of this experiment. Two of our subjects took part in both experiments. M. J. had an average of 7 stools a

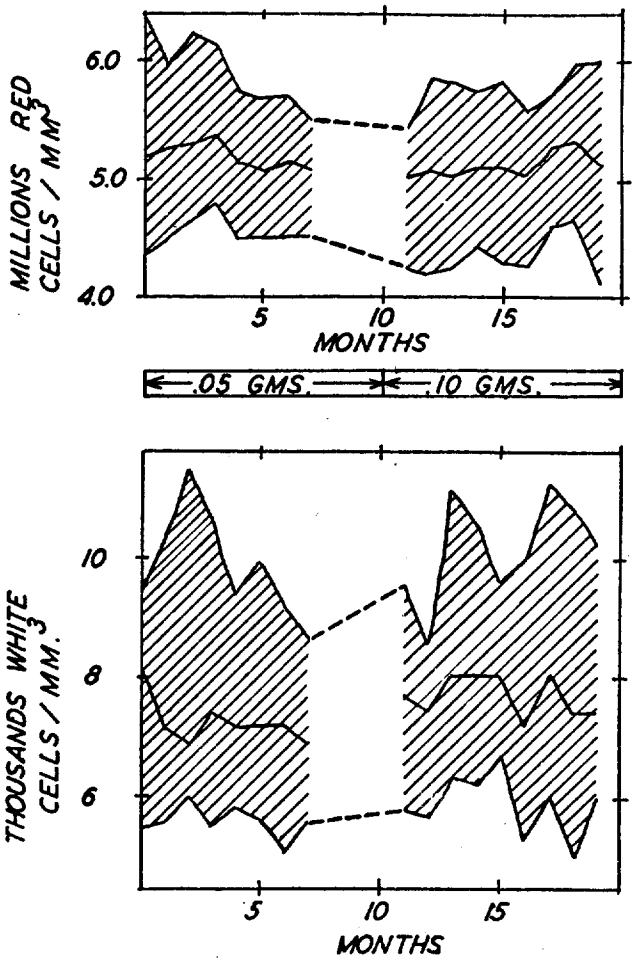

FIG. 3. Red (above) and white (below) blood-cell counts of 11 human subjects ingesting .05 or .10 gram of guaiacum per day.

Upper line: Highest figure observed in any subject each month.
Lower line: Lowest figure observed in any subject each month.
Middle line: Average for the entire group of 11 subjects.
Shaded area: Range within which all observations fell.
Dotted line: No observations made during this period.

week in the former (control) experiment and 7.3 during guaiacum ingestion. V. J. had 8.1 stools in the control period and 7.2 during guaiacum ingestion. As it was also observed in the present experiment that no regular change occurred in the consistency of the

stools, it seems clear that the relatively large daily doses of guaiacum had no ill effect upon colon motility.

The monthly urine-concentration tests were all negative, none of the observations being outside the range for normal kidney function.

No subjective effects, adverse or otherwise, which might be attributed to the guaiacum were noted. The general well-being of all subjects has been unimpaired, although some individuals had the occasional colds to which all are subject.

Fat Utilization and Excretion: An experiment similar to that done upon dogs was carried out on four adult human subjects (three males and one female) on a carefully controlled diet. These indi-

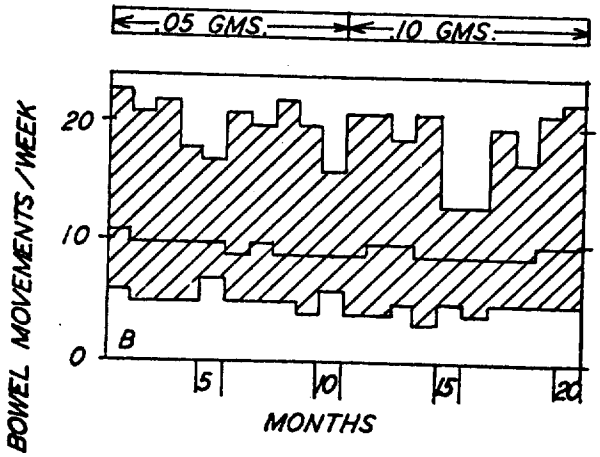

FIG. 4. Number of bowel movements per week for 11 human subjects ingesting .05 or .10 gram of guaiacum per day.

Upper line: Highest figure observed in any subject.
Lower line: Lowest figure observed in any subject.
Middle line: Average for entire group of 11 subjects.
Shaded area: Range within which all observations fell.

viduals ate weighed quantities of food of known composition over a period of eight to 12 weeks. The diet was balanced in all respects and was planned to have a rather high fat content. The daily fat intake varied from 300 grams in some individuals to 600 grams in others. To rule out errors owing to possible inexact knowledge of the fat content of the foods eaten, the following dietary régime was adopted. During the first (control) week an accurate record of the quantity of all materials ingested was kept. During the following week each meal was an exact duplicate, qualitatively and quantitatively, of the corresponding meal of the previous control week. Also, during the second week 100 mgm. of guaiacum per day were taken, dissolved in the daily milk-cream mixture which constituted

the largest single source of fat in the diet. If the diet was changed slightly the third (control) week, the same change was made during the fourth (guaiacum-ingestion) week, etc.

Before the first meal and after the last meal of each four-day period, powdered charcoal was eaten to mark off the feces of the experimental period. The feces were collected for the whole four-day period and analyzed for fat.

The data show that during the control weeks (21 duplicate determinations) the subjects excreted $2.55 \pm .07$ per cent of the fat fed. During the guaiacum-ingested weeks (20 duplicate determinations) $2.76 \pm .10$ per cent of the fat fed was not used. The difference is statistically insignificant. These figures correspond very well with the results of Smith, Miller, and Hawk (1915) who determined that in normal humans, 96 to 100 per cent of fed lard was digested and absorbed.

Our observations on human beings in the chronic experiment indicate that no tendency toward diarrhea is induced by guaiacum ingestion. A further check on this was made in the carefully controlled human dietary experiment reported here by determining the water content of the feces as well as the fat content. During guaiacum-ingestion periods the feces contained $80.7 \pm .8$ per cent moisture as compared with $79.7 \pm .8$ per cent during control periods.

SUMMARY AND CONCLUSIONS

The above extensive work, carried out upon rats, dogs, cats, and human beings over a period of four years, has demonstrated the following with regard to the ingestion of guaiacum in the quantities indicated in the test.

1. In the alimentary canal guaiacum exerts no irritating action, and only in far greater quantities than would ever be ingested is there slight cathartic effect. These results were obtained by analyses of the water content of the feces in experiments on seven dogs, 40 rats, and four human beings and by records of number of weekly bowel movements in 14 human beings during a two-year ingestion experiment as well as by anatomical (gross and microscopic) observations on rats, cats, and dogs.

2. *In vitro* studies testing the digestibility of lard by pancreatic lipase plus bile (161 tests) indicated that addition of guaiacum to the lard had no effect upon the hydrolysis.

3. *In vivo* studies upon the content of unused fat excreted in the feces of 40 rats, seven dogs, and four human beings confirmed the *in vitro* studies indicating the lack of any impairment of fat digestibility or absorption as a result of the presence of guaiacum in lard.

4. Numerous acute and chronic experiments on rats, dogs, and man indicated that ingested guaiacum may remain in the alimentary canal several days and that most of it appears in the feces. *In vitro* studies demonstrated that the guaiacum which failed to be excreted was probably destroyed or changed in the colon. Gastric and pancreatic digestion did not destroy guaiacum. Very little if any of the material is absorbed into the blood. Acute experiments (31) on intravenous ingestion in dogs indicate that the presence of guaiacum in the blood is not injurious.

5. Experiments on 11 dogs, eight cats, and 14 human beings ingesting guaiacum for one to two years showed no effect upon body weight.

6. The blood picture (red and white cell numbers and hemoglobin concentration) of seven dogs and 14 human beings was unaffected by eating guaiacum over long periods of time.

7. The kidney function of 14 human subjects was unchanged during one to two years of daily guaiacum ingestion.

8. Experiments on 40 growing rats over a period of 40 weeks showed that gram for gram lard containing more than enough guaiacum to prevent rancidity is of as great nutritive value as lard free from guaiacum.

9. Growth, reproduction, and longevity of three generations of rats (120 in all) were unaffected by ingesting 100 times as much guaiacum as was necessary to prevent rancidity of the lard fed.

10. Studies upon the microscopic appearance of the intestines, liver, spleen, and kidney of 23 rats, five cats, and seven dogs, and upon the gross appearance of the organs of many other rats revealed that no pathology was caused by prolonged ingestion of large quantities of guaiacum.

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The Pharmacological Evaluation of Antioxidants

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CONTENTS

I. Introduction

Investigations into the problem of rancidity and methods for stabilizing edible oils and fats have been quite extensive. One of the fundamental problems yet to be solved is the mechanism by which fats become rancid and the role of antioxidants in the prevention or delay of rancidification. Mattill (1947) has reviewed the chemical aspects of the problem. The influence of lipoxidases, yeasts, molds, or bacteria on the decomposition of glycerides of fatty acids has also not been clearly elucidated although it is known that the presence of such entities is not necessary for the production of rancidity.

The importance of preserving the wholesomeness of edible fats is exemplified by the fact that in the United States the average individual derives about one-third of his total caloric requirements from fat. Fat not only functions as a concentrated foodstuff, but also has an important relationship to the availability and utilization of essential dietary substances. Some of these substances are vitamin A, carotene, vitamin E, vitamin D, pantothenic acid, pyridoxine, biotin, ascorbic acid, and linoleic acid (Burr and Barnes, 1943). Considerable experimental evidence is available also which emphasizes that the diet of man and animals is enhanced in some way by the addition of fresh natural edible fats. The presence of rancid fats in the diet, however, has a serious deleterious

effect, which goes beyond the destruction of essential dietary constituents. Fitzhugh *et al.* (1944) have demonstrated that the feeding of rancid lard to rats at a dietary level of 6% resulted in a marked emaciation of the animals by the end of the first year on the diet. A progressive type of paralysis was also noted in these animals at the time of termination of the experiments (2 years). The pathological examination verified the fact that the major symptoms were the result of a vitamin E deficiency, although the byproducts of the oxidized fat appear to have a toxic action of their own (Burr and Barnes, 1943).

From these considerations the indications are that considerable emphasis must be placed on the importance of keeping edible fats palatable and wholesome, especially when storage is necessary. Stabilization of fats has been accomplished by several means, such as choice of raw materials, hydrogenation, proper packaging, deodorization, and exercising every precaution during processing to avoid unnecessary exposure to heat and light, or contamination with prooxidants. All these methods have their limitations in that their applications are not always feasible to all fats and fatty food products; hence reliance for the stabilization of fats has been placed on antioxidants. By the addition of traces of certain chemicals it has been found that oxidative rancidity can be inhibited and the age resistance of fats considerably increased. This phenomenon has been known for more than 100 years and the first utilization for prevention of rancidity was in the pharmaceutical field—employment of gum benzoin to protect lard. This product has been official in the United States Pharmacopoeia since 1880.

Many compounds have been found to possess antioxidant properties, the more common compounds being gum guaiac, gallic acid, catechol, and hydroquinone. Numerous other chemicals of the phenolic and acidic type have also had their proponents. Naturally occurring antioxidants have received considerable attention, the purpose being to develop a nontoxic substance suitable for food use. Lecithin, cephalin, tocopherols, and products obtained from cereals, yeasts, sugars, and other food substances have been proposed. Lundberg (1947) has compiled a list of antioxidants which are in current use or which have been proposed for use in stabilizing edible fats.

The criteria which are considered necessary for establishing the usefulness of a chemical as an antioxidant have been listed by Higgins and Black (1944). According to these authors, the antioxidant should be soluble in fats and impart no foreign color, odor, or flavor to the fat even on long storage and should be an effective inhibitor in the treated product for at least a year after storage at a temperature of 75°–85° F. in unsealed containers. The antioxidant should be unchanged when

heated and should possess the ability of retarding rancidity in baked foods prepared from fats treated with the antioxidant. The safety of the antioxidant for food use must be established. There are many antioxidants which are able to pass the physical and chemical criteria for practical usefulness, but to establish proof of nontoxicity is often difficult.

Antioxidants are chemical preservatives and come under the terms of the Federal Food, Drug and Cosmetic Act when they are present in food shipped in interstate commerce. Any antioxidant which may be legally incorporated into foods must be declared on the label in a way to show it to be a preservative. If the chemical is safe and nonpoisonous, it can be incorporated into unstandardized foods as a preservative if it does not conceal damage or inferiority or make the product appear better or of greater value. It may be used in standardized food only if the standard recognizes the preservative as a legitimate ingredient. If the food is not a standardized item and the antioxidant is nonpoisonous, the only requirement is the declaration of the preservative on the label. If the antioxidant is a poisonous and deleterious substance it must be shown that the preservative is required and cannot be avoided in good manufacturing practice. In this event, the law provides that the Administration shall promulgate regulations limiting the quantity of the substance a food may contain so that it will not constitute a hazard to the public health. It is the manufacturer's and shipper's responsibility to secure adequate evidence of safety for the antioxidant that he intends to use. A general plan for the pharmacological investigation, which permits a thorough appraisal of the toxicology of an antioxidant, has been presented (Lehman, 1948).

Of the many chemicals proposed for antioxidant use and under active investigation, pharmacological data on the compounds listed below have become available.

1. Nordihydroguaiaretic acid
2. Propyl gallate
3. Hydroquinone
4. l-ascorbyl palmitate
5. *d*-isoascorbyl palmitate
6. *d*-isoascorbic acid
7. Thiodipropionic acid
8. Dilauryl thiodipropionate
9. Distearyl thiodipropionate
10. Gum guaiac

The palmitates and *d*-isoascorbic acid are not considered very effective as antioxidants and any discussion of their pharmacology will be incidental only. Phenol and catechol are included for the purpose of comparison, although they are too toxic for food use.

II. Acute Toxicity

A summary of the acute toxicity of the antioxidants is presented in Table I. From a practical standpoint it may be stated that an antioxidant which possesses an LD_{50} (the quantity of the antioxidant which is fatal to 50% of the test animals following oral administration) of 1,000 mg./kg. or above has met at least one important criterion of harmlessness. An inspection of the table reveals that nordihydroguaiaretic acid, propyl gallate, thiodipropionic acid and its derivatives, *d*-isoascorbyl palmitate, gum guaiac, and butylated hydroxy anisole have measured up to this criterion. It is of interest to note that in general hydroquinone and phenol have the same order of toxicity.

Table I

The Acute Toxicity of Antioxidants

Animal	Route of administration	Approximate LD_{50} mg./kg.	Source
		Nordihydroguaiaretic acid	
Mice	Oral	>2000	Bieter (1949)
Mice	Oral	4000	F & D A* (1950)
Rats	Oral	>2000	Bieter (1949)
Rats	Oral	5500	F & D A* (1950)
Guinea pigs	Oral	850	Bieter (1949)
Mice	Intraperitoneal	550	Bieter (1949)
		Propyl gallate	
Mice	Oral	2000	Boehm and Williams (1943)
Mice	Oral	3500	F & D A* (1950)
Rats	Oral	3800	Orten *et al.* (1948)
Rats	Oral	3600	F & D A* (1950)
Rats	Intraperitoneal	380	Orten *et al.* (1948)
		Thiodipropionic acid	
Mice	Oral	>2000	Hazleton (1949)
Mice	Oral	2000	F & D A* (1950)
Mice	Intraperitoneal	250	Hazleton (1949)
Mice	Intravenous	175	Hazleton (1949)
Rats	Oral	>2000	Hazleton (1949)
Rats	Oral	3000	F & D A* (1950)
Rats	Intraperitoneal	700	Hazleton (1949)
Rats	Intravenous	>300	Hazleton (1949)
		Dilauryl thiodipropionate	
Mice	Oral	>2000	Hazleton (1949)
Rats	Oral	2500	Hazleton (1949)
Mice	Intraperitoneal	>2000	Hazleton (1949)

TABLE I (*Continued*)

Animal	Route of administration	Approximate LD_{50} mg./kg.	Source
		Distearyl thiodipropionate	
Mice	Oral	>2000	Hazleton (1949)
Rats	Oral	>2500	Hazleton (1949)
Mice	Intraperitoneal	>2000	Hazleton (1949)
		d-Isoascorbyl palmitate	
Mice	Oral	>8000	F & D A* (1950)
Rats	Oral	6000	F & D A* (1950)
		Gum guaiac	
Mice	Oral	>2000	Bieter (1949)
Rats	Oral	>2000	Bieter (1949)
Rats	Oral	>5000	F & D A* (1950)
Guinea pigs	Oral	1120	Bieter (1949)
Mice	Intraperitoneal	>2000	Bieter (1949)
		Catechol	
Mice	Oral	260	Bieter (1949)
Rats	Oral	260	Bieter (1949)
Guinea pigs	Oral	210	Bieter (1949)
Mice	Intraperitoneal	190	Bieter (1949)
		Butylated hydroxy anisole	
Mice	Oral	2000	F & D A* (1950)
Rats	Oral	2200	F & D A* (1950)
		Hydroquinone	
Mice	Oral	400	F & D A* (1950)
Rats	Oral	320	F & D A* (1950)
Guinea pigs	Oral	550	F & D A* (1950)
Pigeons	Oral	300	F & D A* (1950)
Dogs	Oral	200	F & D A* (1950)
Cats	Oral	70	F & D A* (1950)
Rats	Intravenous	115	F & D A* (1950)
		Phenol	
Mice	Oral	520	Bieter (1949)
Mice	Oral	395	F & D A* (1950)
Rats	Oral	440	Bieter (1949)
Rats	Oral	450	F & D A* (1950)
Mice	Intraperitoneal	360	Bieter (1949)

* Food and Drug Administration, unpublished data.

III. Chronic Toxicity

1. Rats

Lifetime feeding studies in rats have been carried out with all the compounds listed. One of the criteria for establishing the harmlessness of a substance which may be ingested chronically when added to food is the effect of such a substance on the growth rate of rats. The first six

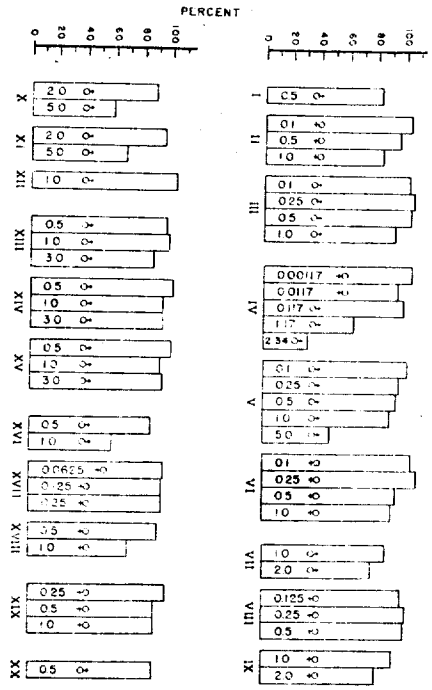

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FIG. 1. Mean growth rate of rats fed the antioxidants for 6 months expressed as per cent of the control groups. The growth rate of the control animals was taken as 100 per cent. The figures in each column represent the per cent of the antioxidant in the diet. The symbol ♂ = male; ♀ = female. The Roman numerals identify the antioxidants and source of the data as follows: I, II, Nordihydroguaiaretic acid (Bieter); III, Nordihydroguaiaretic acid (F&DA*); IV, Propyl gallate, (Orten *et al.*); V and VI, Propyl gallate, (F&DA*); VII, VIII, IX, Hydroquinone, (F&DA*); X, *l*-ascorbyl palmitate, (F&DA*); XI, *d*-isoascorbyl palmitate, (F&DA*); XII, *d*-isoascorbic acid, (F&DA*); XIII, Thiodipropionic acid, (Hazleton); XIV, Dilauryl thiodipropionate, (Hazleton); XV, Distearyl thiodipropionate, (Hazleton); XVI, XVII, XVIII, Catechol, (F&DA*); XIX, Phenol, (F&DA*); XX, Gum guaiac, (Bieter).

* Food and Drug Administration, unpublished data.

months in the life of a rat is usually taken for observing growth changes for the reason that this time period represents the rapidly growing stage. Figure 1 represents a summary of the data of the various compounds. Practically all of the antioxidants are recommended for use in quantities not to exceed 0.01% in animal fats and shortenings. In assessing the harmlessness of the antioxidants, a factor of safety of at least 100 is taken. This requires that when the antioxidant is fed to experimental animals in their total diet at a level 100 times that proposed in fats for human consumption, no significant effect on growth should occur. The results in Fig. 1 are unfavorable to nordihydroguaiaretic acid and hydroquinone. Orten *et al.* (1948) noted some stunting in rats on the 1.17% level of feeding propyl gallate. No significant effect at the 1% level was noted in our rats, but retardation was significant at the 5% level. It may be noted in the figure that we have fed all the antioxidants for a lifetime with the exception of thiodipropionic acid and its esters and gum guaiac. This affords an opportunity to compare the growth effects of the various antioxidants on an equal basis. Since it required 5% of propyl gallate to affect growth in our experiments, it is believed that species difference or some other variable was responsible for the stunting of growth in the 1.17% level of the antioxidant as observed by Orten *et al.*

The dilauryl and distearyl esters of thiodipropionic acid are recommended for use in a concentration of 0.09%. These compounds represent about 0.03% of the acid and since they have been fed at a level of 3.0% without evidence of effect, the 100-times margin of safety appears to have been established.

Gum guaiac is employed in concentrations of 0.1%, and to meet the criterion about a 10% feeding level should have been carried out. Very often technical difficulties do not permit the addition of such high concentrations as would be necessary in the case of gum guaiac to establish a 100-fold margin of safety. This obstacle can be overcome in many instances by supplementary data in other species of animals. This has not been done with gum guaiac, but the long history of its use in medicine without reported injury appears to substantiate the harmlessness of the gum for antioxidant use.

2. Other Species

Chronic studies on propyl gallate have been conducted in guinea pigs and dogs by Orten *et al.* (1948). The guinea pigs were fed at a level of 0.011% of the antioxidant and were observed for one year. No detectable effect on the rate of growth was noted. No influence on reproduction was noted.

A group of 7 dogs was fed propyl gallate for 14 months in amounts

similar to that fed the guinea pigs. The animals on the propyl gallate regimen showed no demonstrable effects and were similar in every respect to the 5 animals which served as controls.

Thiodipropionic acid was fed to guinea pigs by Hazleton (1949) at a level of 0.5% in the drinking water and for a period of 120 days. No significant effect was noted on weight or mortality. Dogs were fed a mixture of 10 parts by weight of dilauryl thiodipropionate and one part of thiodipropionic acid and in a concentration of 0.1% and 3.0% of the acid-ester mixture in the diet. The observation period was 100 days. No untoward effects were noted.

Nordihydroguaiaretic acid has not been subjected to additional studies in dogs which is a serious omission in light of the results in rats. Bieter (1949) employed mice in one series of feeding experiments but the results were inconclusive.

Some observations on the blood constitutents were made by Orten *et al.* (1948) on rats receiving propyl gallate in the diet. The lower levels of antioxidant feeding produced no changes, but concentrations above 1.1% produced significantly lower hemoglobin values. Erythrocite and leucocyte counts were not significantly affected. Hazleton (1949) made similar blood studies on the dogs fed the acid-ester mixture of thiodipropionic acid without finding anything of significance.

In the pharmacological appraisal of an antioxidant it is important to determine the effect of heat on the stability of the substance. Reaction products or a modification of the chemical may occur which may have pharmacological effects. Orten *et al.* (1948) included such experiments in their observations. Rats were fed a heated antioxidant mixture (propyl gallate, lecithin, and corn oil) in an amount at least 100 times that which would be consumed by humans if all the dietary fat were treated with the recommended amount of antioxidant mixture effective in preserving edible fat. No detectable toxic effects were noted. Hazleton (1949) also fed thiodipropionic acid in combination with dilauryl thiodipropionate heated with lard in concentrations at least 10 times that recommended for use as an antioxidant. No injury was noted. We have conducted similar experiments with the ascorbyl palmitates without evidence of injury.

3. Mortality

The mortality rate of rats fed the several antioxidants is also an index of toxicity. A high mortality in any group of treated animals as compared with the controls indicates that the substance under test is quite toxic. If the mortality rate is about equal to that of the controls, the compound may be considered as not highly toxic. Table II summarizes

the available data on the mortality rates of the various compounds. It may be seen that none of the compounds affected the mortality rate of the rats. None of the treated groups showed significant differences from the controls in the number of animals living at any time up to the end of the experiment.

TABLE II

Mortality in Rats after 2 Years of Feeding the Several Antioxidant Diets

Antioxidant	Dosage per cent	Number of Animals Per group	Number of Animals Living at 2 years	Source
Nordihydroguaiaretic acid	0	18	13	Bieter (1949)
	0.1	18	12	
	0.5	18	17	
	1.0	18	12	
Nordihydroguaiaretic acid	0	10	3	F & D A* (1950)
	0.1	10	1	
	0.25	10	3	
	0.5	10	3	
	1.0	10	2	
Propyl gallate	0	16	8	F & D A* (1950)
	0.1	16	4	
	0.25	16	6	
	0.5	16	6	
	1.0	16	4	
	5.0	16	3	
Thiodipropionic acid	0	20	17	Hazleton (1949)
	0.5	20	16	
	1.0	20	13	
	3.0	20	15	
Dilauryl thiodipropionate	0.5	20	10	Hazleton (1949)
	1.0	20	13	
	3.0	20	4	
Distearyl thiodipropionate	0.5	20	18	Hazleton (1949)
	1.0	20	14	
	3.0	20	18	
l-ascorbyl palmitate	0	10	4	F & D A* (1950)
	0.05	10	6	
	0.25	10	4	
d-isoascorbyl palmitate	0.05	10	4	F & D A* (1950)
	0.25	10	5	
d-isoascorbic acid	1.0	10	4	F & D A* (1950)
Gum guaiac	0	10	7	Bieter (1949)
	0.5	10	9	

TABLE II (*Continued*)

Antioxidant	Dosage per cent	Number of Animals Per group	Number of Animals Living at 2 years	Source
Catechol	0	12	2	F & D A* (1950)
	0.0625	12	4	
	0.125	12	4	
	0.25	12	1	
Catechol	0	18	5	F & D A* (1950)
	0.5	18	4	
	1.0	18	4	
Hydroquinone	0	12	2	F & D A* (1950)
	0.125	12	5	
	0.25	12	8	
	0.5	12	8	
Hydroquinone	0	18	5	F & D A* (1950)
	1.0	18	7	
	2.0	18	5	
Phenol	0	12	2	F & D A* (1950)
	0.25	12	5	
	0.5	12	6	
	1.0	12	6	

* Food and Drug Administration, unpublished data.

IV. PATHOLOGY

The predominant pathologic lesions produced by the various antioxidants after lifetime feeding to rats are summarized in Table III. If the criterion of a 100-fold margin of safety on a chronic basis is adhered to, then nordihydroguaiaretic acid and hydroquinone would be considered as deleterious substances for addition to food.

V. SUMMARY

Of the antioxidants listed in the discussion above, evidence of safety for use appears to have been established for propyl gallate, thiodipropionic acid and its dilauryl and distearyl esters, and gum guaiac. Nordihydroguaiaretic acid is not included because no experiments have been conducted in nonrodent species. Hydroquinone must be classed as a harmful and deleterious substance, and as such, has no place as an antioxidant in foods. Catechol has been proposed for antioxidant use (Olcott, 1934), but pharmacological evidence clearly classifies this chemical as a poison.

TABLE III

Pathology in Chronically Poisoned Rats (2-Year Feeding)

Substance	Diet concentration Producing injury	Predominant injury	Source
Nordihydroguaiaretic acid	0.5%	Massive cecal hemorrhages with single and multiple cysts in the mesentery in the angle of junction between small and large intestine	Bieter (1949)
Nordihydroguaiaretic acid	0.5%	Inflammatory cecal lesions and slight cystic enlargement of lymph nodes near the cecum	F & D A† (1950)
Propyl gallate	1.17%	Tubular damage and presence of albuminous casts probably as the result of inanition	Orten *et al.* (1948)
Propyl gallate	5.0%	Patchy hyperplasia in the proventriculus	F & D A† (1950)
Hydroquinone	2.0%	Suggestion of increase in incidence of chronic gastrointestinal ulceration and renal tumors over spontaneous incidence	F & D A† (1950)
* *l*-ascorbyl palmitate	0.25%		
* *d*-isoascorbyl palmitate	0.25%	No lesions present attributable to the compounds fed	F & D A† (1950)
* *d*-isoascorbic acid	1.0%		
* Thiodipropionic	3.0%		
* Dilauryl thiodipropionate	3.0%	No pathological changes	Hazleton (1949)
* Distearyl thiodipropionate	3.0%		
Catechol	0.25%	Beginning hepatic cell hyperplasia	F & D A† (1950)
* Phenol	1.0%	No pathological changes	F & D A† (1950)
* Gum guaiac	0.5%	No damage attributable to the compound	Bieter (1949)

* Highest concentration fed.
† Food and Drug Administration, unpublished data.

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Stabilization of fats and fatty foods

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● ● ● For the stabilization of fats and fatty foods, materials naturally occurring with food products have received most attention since such substances are unlikely to have toxic effects. However, one antioxidant, not occurring with food materials, has been shown to be an effective stabilizer for meat food fats. It is gum guaiac obtained from a tropical tree. It is entirely innocuous physiologically and is a practical commercial antioxidant for lard. It also shows promise for the stabilization of packaging materials for fats and fatty foods and for the stabilization of dehydrated meat products.

FATS and fatty foods are attacked by oxygen, resulting in an oxidative deterioration known as rancidity. Only meager information exists on the reactions that take place. It is well established that they are autocatalytic in nature. They are accelerated by heat, light (especially ultraviolet), and metals such as iron and copper and their salts. The rate is lowered by antioxidants. The refining procedures necessary to produce shortening acceptable to present-day consumers remove large percentages of the natural antioxidants, with the result that refined oils and fats exhibit less resistance to rancidity than do the crude materials.

Oxygen is absorbed by the unsaturated bonds of the fatty acids, with formation of peroxides, and finally certain aldehydes and ketones are produced by rupture of the carbon chain at the oxidized bonds. The degree to which the reaction has progressed determines the extent of the spoilage. There is a latent or induction period of variable length during which small amounts of oxygen are absorbed and only slight organoleptic changes are noted. This is followed by a rapidly accelerated oxygen absorption accompanied by the appearance of the so-called rancid odors and flavors. The final stage is a breakdown of the oxidized bonds, which is accompanied by strong acrid odors.

The logical means of preventing these reactions is either protection from oxygen, as exemplified by vacuum packing, or by the use of antioxidants. The former involves consider-

able difficulty and expense, and does not find application in a wide variety of food products. The use of antioxidants in fats was attempted long ago. Gum benzoin was employed nearly a century ago to prevent rancidity in lard ointments, and the Indians apparently used the bark of certain trees to preserve bear grease (*10*).

Antioxidants naturally occurring with food

No real progress was made in the field of antioxidants until about twenty years ago, when Moureu and Dufraisse (*11*) found that hydroquinone had an inhibitory effect on the oxidation of acrolein and benzaldehyde. Since then a large number of compounds have been shown to possess varying degrees of antioxidant properties for oxidizable materials such as mineral oils, gasoline, rubber, and glyceride oils. Materials naturally occurring with food products have received most attention for stabilization of fatty foods since such substances are unlikely to have toxic effects. The first of these was lecithin, the name applied to the phospholipides of soybean oil, which was proposed by Bollman in 1923 (*1*). It finds some application not only as a stabilizer but also for improving emulsification and frying properties. Carefully purified lecithin possesses no antioxidant activity, and Olcott and Mattill (*20*) showed that the cephalin fraction of the phospholipides carries the inhibitory action.

Portions of the cephalin molecule have been patented separately. The phosphoric acid fragment is covered by Eckey (*5*). Royce (*21*) patented cephalin minus the fatty acid in the alpha position, and Epstein and Harris (*6*) claimed the molecule minus the cholamine and one of the fatty acid radicals. Thurman (*22*) obtained patents on the use of cottonseed and corn oil phospholipides as antioxidants and emulsifiers on the basis that these materials are less likely to oxidize in that they are more saturated than soybean phospholipides.

Certain plant pigments have been shown to be antioxidants. Newton (*14*) indicated that carotenoid pigments, or some material closely associated with them in nature, have antioxidant properties under certain conditions, and that the stability carried through into the baked goods made from the fats. The work of Olcott and Mattill (*19*) showed carotene to be a pro-oxidant. The difference in results lies in the fact that carotene retards oxidation after the induction period has run its course, and the latter authors were concerned with the induction period only, while Newton considered the whole course of the reaction.

Other materials associated with vegetable oils which have received attention as antioxidants are the "inhibitols" of Olcott and Mattill (*18*). These are materials, nonsterol in nature, which are concentrated in the unsaponifiable fraction and which depend for their activity on free hydroxyl groups. Later it was shown that pure tocopherols possessed marked antioxygenic activity (*17*). Recently Olcott concluded that some, if not most, of the antioxidant properties of unsaponifiable fractions of vegetable oils is due to tocopherols (*16*). Tocopherols are effective in lard and purified esters of fatty acids but not in vegetable oils. Golumbic (*7*) recently showed that chroman and coumarin derivatives having a hydroxyl group but no aliphatic side chains are effective antioxidants.

The fractions molecularly distilled from vegetable oils and patented by the Eastman Kodak Company (*4*) contain large percentages of tocopherols.

Grettie (*9*) showed that hydrogenated sesame oil has antioxidant properties in lard and vegetable oils. Wheat germ oil, which probably owes its effect to the synergistic action of tocopherols and phospholipides, has been proposed.

Turning from the materials associated with oils, we note that the Musher Foundation (*13*) has supported a considerable amount of study on the practical application of oat flour as an antioxidant. It has been suggested for the stabilization of butter, ice cream, potato chips, and many other fat-containing foods, as well as for packaging materials for them. The nature of the oat flour antioxidant is not definitely known, although it is claimed to be a protein-fat complex (*3*). More recently the Musher Foundation (*13*) obtained patents on the use of many substances and combinations of substances as antioxidants. Among these are preparations from cereals, sugars, grains, milk solids, oils, yeast, animal tissues, legumes, and grasses.

The dicarboxylic acids occurring in fruits were investigated by Greenbank and Holm (*8*). They found maleic, tartaric, and citric acids to be effective in lard and vegetable oils. It has since been established that, to be effective, those acids containing more than three carbons must also contain a hydroxyl group.

Combinations of antioxidants have been shown to give protection in excess of that expected from the results with either one alone (*16*). Combinations of inhibitols with acids and of phenols with acids are especially effective.

One antioxidant, not occurring with food materials, has received considerable attention. It is gum guaiac, proposed by Newton and Grettie (*15*). It is obtained from a tropical tree, *Guaiacum officinalis*, which grows in Central America and the West Indies. Extensive tests carried out by Carlson at the University of Chicago have proved it to be entirely innocuous physiologically (*2*). It is effective in meat food fats but shows only slight antioxidant activity in vegetable oils. Its stabilizing effect carries through into the baked goods prepared from the fats.

Table I gives comparative results obtained in this laboratory with several antioxidants on lard and cottonseed oil. Pyrogallol is the most effective in lard, while citric acid has the greatest effect on cottonseed oil. The combination of gum guaiac and phosphoric acid produces remarkable stability. This substantiates earlier results on the synergistic effect of phenols and acids.

Table I. Comparative Antioxidant Properties

	Hr. by Active Oxygen Method	
	Lard	Cottonseed oil
Control	2	12
Control + 0.01% pyrogallol	10	14
Control + 0.002% phosphoric acid	6	18
Control + 0.002% citric acid	8	20
Control + 0.02% wheat germ oil	20	14
Control + 0.01% lecithin	11	12
Control + 0.01% inhibitol	8	14
Control + 0.002% gum guaiac + 0.002% phosphoric acid	25	15
Control + 0.02% gum guaiac + 0.02% citric acid	3	40
Control + 0.02% gum guaiac + 0.02% phosphoric acid	44	50

Gum guaiac

During two years of commercial use, gum guaiac has proved to be a practical and effective stabilizer for lard. It has been used in the stabilization of a highly processed (bland) lard, which without the protection of guaiac would have a stability of 3 to 5 hours by the active oxygen method. The lard treated with 0.05 per cent guaiac ranges from 15 to 25 hours in stability. Samples remain in good organoleptic condition for over a year at room temperature. Crackers prepared from the stabilized lard keep 16 to 20 days at 140° F. as compared to 2 to 4 days for crackers made from unstabilized processed lard.

The increase in lard stability brought about through the use of gum guaiac may be of great commercial importance to

the producer as well as to the consumer. Since ordinary packaged lard must be held under refrigeration, it can be displayed and sold only on the meat counters. The stabilized lard is handled on the grocery shelves along with other shortenings. Stabilization with an antioxidant rather than by hydrogenation retains all of the excellent nutritional properties, such as the high digestibility and essential fatty acid content of lard.

Gum guaiac is easily incorporated into fats even though it is not readily soluble in them. It can be added to the steam or dry-rendering tanks during the rendering period. It can also be incorporated into the fats after rendering by the use of a mutual solvent—that is, one which will dissolve the gum and, in turn, dissolve in the fat. The gum is dissolved in the solvent and filtered to remove the solid material consisting of small particles of sand, bark, and wood which the crude material usually contains. The solution is then added to the fat, preferably as it is maintained under a vacuum and at a temperature sufficiently high to vaporize the solvent.

Gum guaiac has utility for the stabilization of animal fats during storage. The keeping qualities of good lard, for example, may drop 50 to 100 per cent during a storage period of 6 months to 2 years. Guaiac-treated lard going into storage with a stability of 20 to 25 hours will have a much better stability at the end of the storage period than untreated lard entering storage with a stability of 8 to 12 hours.

Interesting results have been obtained on the stabilization of other meat food fats with guaiac. Results obtained with oleo oil and on crackers made from it are as follows:

	Hr. by Active Oxygen Method	Days before Crackers Are Ranced at 140° F.
Original Oleo oil	9	27
Original + 0.1% gum guaiac	52	52

This antioxidant also has good stabilizing properties in chicken fat. Results obtained on addition of the guaiac after rendering, as well as during the rendering period are as follows:

	Hr. by Active Oxygen Method
Rendered chicken fat	22
Same + 0.1% gum guaiac	35
Chicken fat rendered with 0.1% gum guaiac	100

The present nonavailability of tinware for food packaging has resulted in the introduction of a number of dehydrated foods. It is the consensus in many quarters that certain dehydrated products may continue in demand after the war. Dried soups have already reached a sizable volume. Scarcity of shipping space has made necessary the dehydration of meats for shipment abroad and for concentrated army rations. Orders have already been placed for quantities of dehydrated beef, and it is said that dried pork will be produced in much larger volume.

Gum guaiac has a probable further application in these dehydrated foods. The fats in such foods tend to turn rancid and thus harm the qualities of the products. The stabilizing effect of guaiac on chicken fat has already been indicated. Tests now underway show that some increased keeping quality is imparted to dehydrated beef by guaiac, and that the storage life of dehydrated pork is increased markedly. Gum guaiac is heat stable and, therefore, withstands the cooking and drying processes used in the preparation of the dehydrated materials.

Another application of gum guaiac is the stabilization of paper packaging materials for fats and fatty foods. This takes on an added importance during the present shortage of metals for food packages. It can be incorporated into certain papers during their manufacture. One application is in liners for lard and shortening cartons. Here the thin layer of fat which is absorbed by the liner and carton quickly becomes rancid (in 3 months at 75° F.). The presence of the antioxidant in the paper liner and carton retards this appreciably (to 6 months at 75° F.). Experiments are underway on various other packaging materials for meats, poultry, and dairy products.

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Picking Prunes for Dehydration

(See article on page 53)